COK-HOL307-1-P024-165

The American Journal of Medicine

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Volume XIX

JULY TO DECEMBER

1955

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NEW YORK MCMLV

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The American Journal of Medicine

VOL. XIX JULY, 1955 No. 1

Editorial

Subacute Bacterial Endocarditis: Optimal Duration of Treatment

AFTER a dozen years' experience with antibiotic therapy of subacute bacterial endocarditis certain points have been established: (1) Penicillin is usually much more effective than other antibiotics. (2) Moderately large doses should be given. (3) The period of chemotherapy must be of some length. The aim of this editorial is to consider the evidence bearing on the last point, the optimal duration of antibiotic treatment.

It may be recalled that when penicillin first became available, distribution to physicians in the United States was the responsibility of the National Research Council's Committee on Chemotherapy. In view of great demand and limited supply, this Committee released penicillin with the recommendation that specific doses be employed. Initially the quantity allocated for treatment of a case of bacterial endocarditis was 4,200,000 units, to be given either at the rate of 300,000 units daily for fourteen days or 200,000 units daily for twenty-one days. These schedules of treatment achieved successful results in at least half of the patients treated.[1] Soon it became apparent, however, that some infections required more intensive therapy; consequently, as supplies became more plentiful there was a tendency to give larger doses for longer periods of time.

In 1948 Keefer presented to the Association of American Physicians an analysis of the results of 450 cases collected by the Committee on Chemotherapy, together with 26 cases personally observed.[2] The following two sentences are taken from that report: "It has been demonstrated that the largest number of patients (75 to 80 per cent) recover following an initial course of 500,000 units of penicillin daily for eight weeks. When less than 500,000 units are given daily, and treatment is continued for less than eight weeks, then the relapse rate is high and the number that recover is decreased (60 to 70 per cent)." This statement undoubtedly has had considerable influence on our practice in the treatment of bacterial endocarditis during the succeeding years. Many physicians believe that the safest course is to continue chemotherapy in all cases for six to eight weeks. Finland, in a recent comprehensive review,[3] accepts the view that "large doses must be given continuously over long periods," and states further that, "although large doses given for short periods have sometimes proved effective and have been suggested as a useful form of administration, this method has also been tried and found wanting in many cases." The evidence cited consists of the reports of Hamburger and Stein[4] and of King et al.,[5] both of which will be discussed here in some detail.

There is no doubt that some cases of subacute bacterial endocarditis can be cured by comparatively short courses of penicillin therapy. On various occasions treatment had been discontinued after only ten to fourteen days for one reason or another, yet the infection was found to

[1] MEADS, M., HARRIS, W. and FINLAND, M. The treatment of subacute bacterial endocarditis with penicillin. *New England J. Med.*, 232: 463, 1945.

[2] KEEFER, C. S., ANDERSON, D. G. and HEWITT, W. L. End results in the treatment of bacterial endocarditis. *Tr. A. Am. Physicians*, 61: 112, 1948.

[3] FINLAND, M. Treatment of bacterial endocarditis. *New England J. Med.*, 250: 372, 1954.

[4] HAMBURGER, M. and STEIN, L. Streptococcus viridans subacute bacterial endocarditis. Two week treatment schedule with penicillin. *J. A. M. A.*, 149: 542, 1949.

[5] KING, F. H., SCHNEIERSON, S. S., SUSSMAN, M. L., JANOWITZ, H. D. and STOLLERMAN, G. H. Prolonged moderate dose therapy versus intensive short term therapy with penicillin and caronamide in subacute bacterial endocarditis. *J. Mt. Sinai Hosp.*, 16: 35, 1949.

have been eradicated. In 1949 Hamburger and Stein reported their experience with twelve patients each of whom had been treated for two weeks, 15,000,000 or 16,000,000 units of penicillin being given daily either by continuous intravenous injection or by intermittent intramuscular injection. There were two deaths in the series, neither one due to continuing infection; two patients had relapses within one month but were successfully retreated according to the same schedule; the remaining eight had been cured by the first course of treatment. Hunter in 1952 mentioned five patients, all of whom had responded satisfactorily to treatment consisting of 2,500,000 units of penicillin and 2 gm. of streptomycin daily for only ten days.[6]

Hunter had shown by laboratory experiment that streptomycin enhances the bactericidal effect of penicillin on enterococcus and on some of the other nonhemolytic streptococci. Robbins and Tompsett confirmed the effectiveness of this combination in the therapy of enterococcus infection.[7] Although its value is less certainly established in endocarditis caused by other kinds of streptococci, many clinicians have adopted the practice of employing it in all cases involving nonhemolytic streptococci.

Let us now examine the main pieces of evidence that have provided the basis for belief that long courses of therapy are safest for the treatment of all cases.

1. The conclusion of Keefer and his associates, based on analysis of the early cases treated under supervision of the Committee on Chemotherapy, has already been mentioned. It should be noted that the data were obtained during early experiences with penicillin when doses were small, as measured by present standards, and that the combination of streptomycin with penicillin was not then being used.

2. Cates and Christie in 1951 reviewed their experiences with more than 400 patients treated in Great Britain under the auspices of the Medical Research Council.[8] In early trials an experiment to assess the importance of duration of treatment was conducted on three groups of patients, all of whom received a total dose of 5,000,000 units. Those in one group were given 1,000,000 units daily for only five days; treatment failed in 83 per cent. Patients in the second group received 500,000 units daily for ten days; 50 per cent were failures. Those in the third group received 250,000 units daily for twenty days and only 22 per cent were failures. This demonstrates clearly the importance of duration of therapy in determining outcome; however, treatment periods employed in the early trials may not be applicable to present-day practice because of the small amounts of penicillin given and because streptomycin was not being used as an adjuvant.

3. The clinical experiment of King et al.,[5] carried out in 1948, is of great interest and its result also has had considerable influence. The purpose of the experiment was to determine the effectiveness of massive antibiotic therapy given for a period of ten days. Eight patients infected with organisms sensitive to penicillin were included in the study. Each one received ten rapid intravenous injections of 1,000,000 units of penicillin at hourly intervals each day; during the remainder of the twenty-four hour period four additional intramuscular injections of 1,000,000 units were given. Thus the total quantity of penicillin administered to each patient was 14,000,000 units per day. In order to increase the already high tissue levels of penicillin, caronamide was given. The results obtained were extraordinarily poor; only one of the eight patients was cured, yet four of the remaining seven later responded satisfactorily to treatment consisting of 400,000 to 900,000 units daily for five or six weeks. The authors discussed possible reasons for the failure of the intensive course of therapy and reached the conclusion that the short duration of treatment was the principal factor. Other writers have agreed.[3,8]

However, another possibility was mentioned by King et al., namely, that the penicillin levels obtained may have exceeded the concentration which provides for optimal bactericidal effect. They refer to Eagle's preliminary report that such a paradoxical "zone" effect can be demonstrated *in vitro*. Eagle has since amplified his observations,[9,10] and there is some basis for the

[6] HUNTER, T. H. The treatment of some bacterial infections of the heart and pericardium. *Bull. New York Acad. Med.*, 28: 213, 1952.

[7] ROBBINS, W. C. and TOMPSETT, R. Treatment of enterococcal endocarditis and bacteremia. *Am. J. Med.*, 10: 278, 1951.

[8] CATES, J. E. and CHRISTIE, R. V. Subacute bacterial endocarditis. *Quart. J. Med.*, 20: 93, 1951.

[9] EAGLE, H. and MUSSELMAN, A. D. The rate of bactericidal action of penicillin in vitro as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. *J. Exper. Med.*, 88: 99, 1948.

[10] EAGLE, H. Further observations on the zone phe-

belief that this may explain failure of treatment in many of the cases under consideration. Eagle showed that when the concentration of penicillin in a medium is elevated beyond a certain zone the bactericidal effect is sharply curtailed. He found also that the diminution of the bactericidal effect of too high concentrations persists for hours after the organisms have been transferred to a medium containing quantities of antibiotic optimal for bactericidal action.

Thus these patients might not have benefited from the more conventional dosage schedule during that part of each day when the intramuscular injections were being given. Also noteworthy is the fact that the only patient whose infection was eradicated initially was the one harboring the organism most resistant to penicillin. The fact that some cases of bacterial endocarditis have required treatment with doses of penicillin as high as or higher than 14,000,000 units per day does not invalidate this hypothesis since in such cases the high dosage has only been resorted to after smaller quantities have been found ineffective, indicating considerable resistance on the part of the infecting organism. The "zone" phenomenon has generally been regarded as having little clinical significance. In the case of bacterial endocarditis, however, we are dealing with an infection in which such an effect might be anticipated because bactericidal action of the chemotherapeutic agents appears to be all-important.

It would appear, then, that we are now justified in re-opening the question of necessity for prolonged antibiotic treatment in all patients with this disease. Some tendency to shorten the average course of therapy is already evident. Reports on treatment of bacterial endocarditis from various clinics indicate that discontinuance of treatment at the end of twenty-one to thirty days is not unusual. Geraci states that fifteen selected patients, all with penicillin-sensitive organisms, were treated for only ten to fourteen days with a penicillin-dihydrostreptomycin combination and satisfactory results were obtained.[11] Review of our experience at the New Haven Hospital in the past two years, during which the penicillin-streptomycin combination was used in all cases, reveals that only two of eighteen patients were treated for as long as forty-two days, therapy being stopped in twenty-one to thirty days in fifteen, and after fourteen days in one patient. There is no record that relapse of the infection occurred in any of these patients.

It is probable that selection can be made of patients who may be expected to respond satisfactorily to treatment maintained for only two to four weeks. Two principal criteria for such classification would be short duration of illness and an infecting organism sensitive to penicillin. A further indication would be prompt clinical improvement after the inception of antibiotic treatment. Conversely, there are criteria which indicate advisability of prolonged treatment. These include: (1) Long duration of illness before beginning of therapy; it is generally agreed that when symptoms have been present for more than two to three months cure by chemotherapy is considerably more difficult. (2) Infecting organism relatively insensitive to penicillin, as in the case of enterococcus infection. (3) Patients without demonstrable bacteremia; diagnosis may be delayed in such cases, and the effect of penicillin may be impaired by the fact that colonies of bacteria are situated deep in the vegetations, inaccessible to both antibiotics and phagocytes.

The physician who accepts responsibility for management of a case of bacterial endocarditis must maintain a close follow-up for evidence of relapse, particularly during the first month or two. A relapse, while certainly not desirable, is not necessarily disastrous, as re-treatment may yet be successful. There is not much likelihood that significant alteration in penicillin-sensitivity will occur. In a disease of such gravity conservatism is certainly proper, and all would agree that any error should be in the direction of overly long treatment. Nevertheless, there are grounds for suggesting that in selected cases antibiotic therapy can safely be terminated after two to four weeks. This would mean considerable saving to the patients in terms of discomfort and expense.

PAUL B. BEESON, M.D.

nomenon in the bactericidal action of penicillin. *J. Bact.*, 62: 663, 1951.

[11] GERACI, J. E. Antibiotic therapy of bacterial endocarditis. II. Current trends in the treatment of subacute bacterial endocarditis. *Minnesota Med.*, 35: 861, 1952

Clinical Studies

Influence of Gonadal Hormones on Protein-Lipid Relationships in Human Plasma*

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with the technical assistance of Julie Raymunt
New York, New York

EARLIER studies reported from this laboratory have emphasized the influence of sex on protein-lipid relationships in human plasma. Using the Cohn protein microfractionation method, Russ, Eder and Barr[1] have shown that normal young women have a relatively greater amount of alpha lipoprotein and a correspondingly smaller amount of beta lipoprotein than do normal young men, a distinction that is no longer apparent after the period of the menopause. This work has since been confirmed by others with electrophoretic and ultracentrifugal methods.[2–4] Jones and his associates[5] have shown that certain lipoproteins having a density of S_f 10–100 in the ultracentrifuge are in lower concentration in the plasma of young women than in that of young men.

These observations are of considerable interest because of their possible relevance to the pathogenesis of atherosclerosis, and particularly since it is generally known that in young women myocardial infarctions seldom develop.[6–8]

Investigation of the action of gonadal hormones which was begun in our laboratory in 1951 was stimulated by the previous demonstration of sex differences in the distribution of lipoproteins. It was encouraged by the report of Eilert in 1949[9] that estrogens administered to premenopausal and postmenopausal women cause reduction in both cholesterol concentration and cholesterol-phospholipid ratios of plasma. Some preliminary observations from our early studies on the influence of estrogens were published in 1952 and 1953.[10,11] The results obtained from administration of androgens have been mentioned briefly elsewhere.[12] This report is a more detailed presentation of data that have accumulated over a period of three years.

METHODS

Plasma proteins were separated by method No. 10 of Cohn and his associates[13] in which alpha lipoproteins are recovered in Fraction IV+V+VI and beta lipoproteins in Fraction I+III. Analyses for protein, cholesterol and phospholipids were made in unfractionated plasma and in Cohn fractions IV+V+VI, II, and I+III. The methods of fractionation and analyses have been described in a previous paper of this series.[1]

I. Effects of Estrogens

A. Action of Estinyl® (Ethinyl Estradiol) in Survivors of Myocardial Infarction. The first tests of the action of estrogens were made on survivors of myocardial infarction who were selected for two reasons. The group was composed chiefly of males who presumably had minimal amounts of circulating estrogens before treatment. Moreover, previous observations[14] had shown that such patients usually exhibited abnormalities in plasma protein-lipid patterns of a degree that should facilitate detection of any corrective action of the hormones.

For several reasons the estrogen, ethinyl estradiol (estinyl), was chosen for initial trial. Previous experience with its use had been considerable; it could be given by mouth; its toxic

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These studies have been aided by a grant from the U. S. Public Health Service, by support from the Commonwealth Fund and the Albert and Mary Lasker Foundation, and by gifts from Mrs. Katherine Lilly Conroy.
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action had been minor. A daily dose of 1.0 mg. calculated as equivalent to 10,000 rat units[15] was used routinely except as otherwise specified. Choice of this dosage was arbitrary but was based on the intention of making the first tests of action as decisive as possible. When the dose was found to be effective, it was continued with little modification in order to make subsequent observations comparable.

In Table I, the results of the use of estinyl in seven survivors of myocardial infarction have been assembled. All of the patients had had one or more myocardial infarctions.

Before treatment the plasma of all subjects exhibited significant deviations from normal protein-lipid relationships. In two of them the concentration of cholesterol in unfractionated plasma exceeded the normal range. In all the percentage of total cholesterol in Fraction IV+V+VI was lower, and that in Fraction I+III higher, than normal. There was a tendency in the group toward slightly higher than normal cholesterol-phospholipid ratios in the unfractionated plasma and in Fraction I+III.

Following the ingestion of estinyl the abnormalities in protein-lipid relationships were partially or completely corrected. The most constant and characteristic effect was a change in the distribution of cholesterol, with a higher percentage of the total in Fraction IV+V+VI. In the unfractionated plasma, reduction of the concentration of cholesterol was evident in five of the seven patients and was relatively greater than that of the phospholipids. In Fraction IV+V+VI concentrations of cholesterol and phospholipids tended to increase proportionately so that the ratios of the Fraction were not appreciably changed. In Fraction I+III, however, the diminution in the cholesterol concentration so far exceeded that of the phospholipids that the ratios were lowered and in several instances were less than unity.

In inspection of the results, it should be noted that the cholesterol-phospholipid ratios of unfractionated plasma reflect both the altered distribution of lipids and the lower ratio of Fraction I+III. Since both of these factors tend to reduce the ratio in plasma, the change in this value constitutes a good index of the effect of the hormone.

Mean values before and after hormone administration are omitted because variations in dosage, duration of treatment, and other circumstances of the experiments render invalid strict comparison of individual results. At the foot of Table I, however, mean values for young men aged eighteen to thirty-five have been included for convenient comparison with the initial values for the survivors of myocardial infarction and with those obtained following the administration of estinyl.

As previously reported[10] maximum effects of estinyl were attained approximately six weeks following the initial dose of the hormone. Thereafter, the action continued with slight variation but without significant augmentation.

The longest continuous administration of estinyl in amounts of 1.0 mg. daily was approximately six months. When the use of estinyl was continued over long periods without interruption, there was no evidence of diminution of effect, acquisition of tolerance, or of resistance to the hormone.

Changes following withdrawal of the hormone are also illustrated in the table. At first, the concentration of total cholesterol in the plasma and in Fraction I+III rose rapidly and at times exceeded the pretreatment levels. Within a period of two or three weeks, the values returned to a pattern almost identical with that seen before treatment.

Comment: The gonadal effects of the hormone were evident in all cases. Loss of sexual desire and potency were noted early; after three to four weeks, enlargement of the breasts was noticeable and impotence became complete. Usually, the patients were restless, often they were dissatisfied, and they were depressed during the periods when estinyl was administered. In a few instances, gastric distress or nausea was troublesome at the outset of treatment but subsided with its continuance.

In those patients who had experienced anginal pain before the hormonal administration was started, no consistent effect of the hormone could be established. One patient (*Joh*) had an attack of moderately severe pain accompanied by electrocardiographic evidence of myocardial infarction while the hormone was being given. Another (*Gre*) developed a fatal infarction one month after hormonal treatment had been discontinued. It is perhaps worthy of mention that none of these patients of outstandingly poor prognosis died during the long periods of administration of the hormone.

In this study interest was focused upon one group of men who had survived myocardial infarction. Oliver and Boyd[16] have recently

TABLE I

EFFECTS OF ADMINISTRATION AND WITHDRAWAL OF ESTINYL IN CASES OF MYOCARDIAL INFARCTION

Subject, Sex and Age	Date	State	Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III			Remarks
			Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Cholesterol (% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	
Gil M, 39	12/18/51	Estinyl 1.0 mg. o.d. started	300	...	....	27	8.9	...	....	274	...	....	Myocardial infarction in 1949 and in 9/51; a third infarction occurred 10/19/53, at a time when patient was receiving no hormone therapy
	1/18/52		220	312	0.71	41	18.6	91	0.45	176	196	0.93	
	1/31/52		224	309	0.72	43	19.1	107	0.40	179	181	0.99	
	2/21/52		201	279	0.72	54	27.0	114	0.47	144	147	0.98	
	3/6/52	Estinyl discontinued	179	266	0.67	50	28.6	120	0.42	124	139	0.89	
	3/20/52		280	360	0.78	39	14.0	94	0.42	237	242	0.98	
	4/4/52		300	343	0.87	26	8.9	70	0.37	273	262	1.04	
	4/17/52		278	290	0.96	30	10.9	67	0.45	242	199	1.21	
	5/1/52	Estinyl 1.0 mg. o.d. started	276	304	0.91	31	11.1	80	0.39	244	211	1.16	
	5/8/52		209	294	0.71	33	16.1	84	0.39	171	193	0.89	
	5/22/52		190	288	0.66	39	21.3	106	0.37	141	157	0.90	
	6/6/52		206	...	....	43	20.4	...	....	167	...	....	
	7/1/52		171	270	0.63	54	31.4	144	0.37	116	126	0.92	
	7/10/52	Estinyl discontinued	187	...	....	56	30.5	...	....	126	...	....	
Yat M, 49	1/24/52	No treatment	322	...	....	27	8.5	...	....	295	...	....	Myocardial infarction 6/50; thrombophlebitis with pulmonary infarcts 12/51; was receiving anticoagulants during most of period of observation
	2/1/52	Estinyl 1.0 mg. o.d. started	...	...	....	..	....	...	....	...	...	....	
	2/14/52		261	...	....	47	18.3	...	....	207	...	....	
	3/6/52		228	...	....	52	23.4	...	....	170	...	....	
	3/21/52		220	312	0.70	57	25.5	141	0.40	165	164	1.01	
	4/4/52		232	303	0.77	50	22.5	110	0.45	169	153	1.10	
	4/17/52		227	...	....	57	26.0	...	....	162	...	....	
	5/1/52		198	288	0.69	55	29.4	132	0.42	132	136	0.97	
	5/20/52		220	337	0.65	63	29.0	157	0.40	154	165	0.93	
	6/5/52		186	...	....	52	27.9	...	....	132	...	....	
	6/26/52	Estinyl discontinued	...	...	....	..	....	...	....	...	...	....	
	7/20/52		295	...	....	48	16.6	...	....	237	...	....	
	7/24/52		300	341	0.88	38	13.0	86	0.44	248	212	1.17	
	8/7/52		347	370	0.94	38	11.4	115	0.33	290	216	1.34	
	10/23/52	Estinyl 1.0 mg. o.d. started	344	389	0.89	46	13.5	124	0.37	287	270	1.06	
	11/6/52		263	310	0.85	52	19.8	110	0.47	209	196	1.07	
	11/20/52		254	282	0.90	59	23.1	134	0.44	195	166	1.17	
	12/11/52		214	307	0.70	63	30.5	146	0.43	145	147	0.99	
	1/15/53		195	290	0.67	54	28.7	134	0.40	134	147	0.91	

Cre M, 53	1/17/52	Estinyl 1.0 mg. o.d. started	202	...	....	19	9.7	...	....	171	..	....	Myocardial infarction 1/11/52; occasional glycosuria; died of a second myocardial infarction 5/22/53; was receiving no hormones at time of death
	1/30/52		236	...	....	36	15.0	...	....	201	...	....	
	2/5/52		223	...	....	41	18.9	...	....	171	...	....	
	2/14/52		216	280	0.77	39	17.8	86	0.45	176	174	1.01	
	2/20/52		193	256	0.75	43	21.6	98	0.44	154	133	1.16	
	3/27/52		184	266	0.69	54	30.1	121	0.45	122	120	1.02	
	4/10/52		181	272	0.67	58	32.4	130	0.45	120	114	1.05	
	4/24/52		176	254	0.69	51	29.9	121	0.42	119	115	1.03	
	5/8/52		190	290	0.66	54	29.8	140	0.39	128	126	1.02	
	5/22/52		173	281	0.62	50	29.6	137	0.37	120	117	1.03	
	6/5/52	Estinyl dosage reduced to 0.5 mg.	...	...	....	..	....	...	....	...	...	....	
	7/3/52		182	243	0.75	51	27.9	113	0.45	132	124	1.06	
	7/15/52		198	...	....	56	29.5	...	....	135	...	....	
	8/1/52	Estinyl dosage reduced to 0.25 mg.	...	...	....	..	....	...	....	...	...	....	
	8/14/52		180	255	0.71	54	31.6	136	0.40	117	119	0.98	
	9/11/52		205	...	....	64	30.7	...	....	144	...	....	
	9/25/52		217	296	0.73	68	31.2	155	0.44	148	138	1.07	
Sie M, 22	2/4/52	No treatment	218	250	0.87	23	10.9	53	0.43	185	166	1.11	Myocardial infarction 1/14/52; unimpaired health before coronary attack; asymptomatic during observation period
	2/20/52	Estinyl 1.0 mg. o.d started	...	...	....	..	....	...	....	...	...	....	
	3/6/52		179	...	....	54	30.5	...	....	124	...	....	
	3/20/52		172	238	0.72	54	31.8	117	0.46	114	109	1.05	
	3/26/52		223	...	....	..	....	...	....	...	...	....	
	4/3/52		239	260	0.92	69	36.7	110	0.63	160	120	1.36	
	4/24/52		223	...	....	40	18.7	...	....	170	...	....	
	6/3/52		244	231	1.06	48	20.5	91	0.53	178	143	1.24	
Spi M, 29	1/3/52	No treatment	238	270	0.88	27	11.9	72	0.38	201	160	1.26	Obesity; persistent hypertension, 180/120; myocardial infarction 12/28/51; anticoagulants from 12/28/51 to 2/12/52
	1/10/52	Estinyl 1.0 mg. o.d. started	269	271	0.99	36	13.9	66	0.54	219	171	1.28	
	1/30/52		225	280	0.80	43	19.1	101	0.43	177	166	1.07	
	2/5/52		212	299	0.71	52	25.2	113	0.46	151	158	0.96	
	2/18/52	Estinyl discontinued	219	312	0.70	55	25.4	128	0.43	158	171	0.92	
	3/6/52		234	260	0.90	38	16.1	86	0.44	197	162	1.22	
	4/10/52		234	249	0.94	35	15.5	77	0.45	186	136	1.37	
	4/24/52		222	249	0.89	32	14.3	73	0.44	185	151	1.22	
	5/8/52		242	290	0.83	28	11.3	78	0.36	212	197	1.08	
Joh M, 44	9/18/52	No treatment	262	...	....	30	12.9	...	....	193	...	....	Myocardial infarctions in 1945, 1949 and on 1/1/52; chest pain without EKG evidence of infarction; on 1/22/52 had dizziness and pulse irregularity without EKG evidence of another myocardial infarction
	1/7/52		216	270	0.80	29	14.2	63	0.46	171	161	1.06	
	1/17/52	Estinyl 1.0 mg. o.d. started	...	...	....	..	....	...	....	...	...	....	
	1/23/52		216	294	0.73	41	19.6	93	0.44	167	160	1.04	
	1/31/52		209	270	0.77	39	19.2	88	0.44	161	162	0.99	
	2/5/52		224	329	0.68	55	25.9	124	0.44	153	174	0.88	
Min M, 28	11/20/50	No treatment	228	225	1.01	23	11.9	64	0.36	171	130	1.32	Had been followed for 15 yr. as a case of chronic glomerular nephritis with nephrotic syndrome; at time of these observations showed signs of renal insufficiency but no evidence of nephrotic syndrome
	2/28/52	Estinyl 1.0 mg. o.d. started	276	262	1.05	50	18.2	89	0.56	214	142	1.51	
	3/13/52		256	...	....	65	26.6	...	....	177	...	....	
	3/27/52	Estinyl discontinued	273	380	0.72	77	29.1	179	0.43	183	166	1.10	
	4/3/52		332	...	....	76	23.5	...	....	242	...	....	
	4/30/52		290	314	0.92	43	14.9	83	0.52	238	198	1.20	
Mean: 24 normal men (aged 18–35 yr.)			197	228	0.86	49	25.2	100	0.49	142	118	1.20	

reported similar results with the use of only 0.4 mg. estinyl daily in twenty hypercholesterolemic men with coronary heart disease. They noted also that the decrease in concentration of cholesterol was entirely in the esterified fraction and that content of free cholesterol was unchanged by the hormone. In four of the seven cases in which cholesterol partitions were performed, we found that the free cholesterol decreased with the total. The average of the free to total ratios before and after was not significantly altered.

Our observations offer no information concerning the action of estinyl in women. The studies of Eilert[9,17] indicate that similar effects on cholesterol concentration and cholesterol-phospholipid ratios were obtained following the use of estinyl in both pre- and postmenopausal women.

B. Action of Estinyl (Ethinyl Estradiol) in Primary Hypercholesterolemic Xanthomatosis. In the previous group of patients, the occurrence of myocardial infarction was interpreted as clinical evidence of the presence of atherosclerosis. None of the cases, however, was an extreme example either clinically or chemically of predisposition to a deposit of lipids in the arterial walls or in other tissues. To study the effect of estinyl under circumstances in which maximum susceptibility to atheromatous disease is clinically and chemically evident, patients with primary hypercholesterolemic xanthomatosis were selected. Results of seven such cases are presented in Table II. All of them were hypercholesterolemic and exhibited in exaggerated form deviations from normal patterns of protein-lipid relationships. Six of the group gave a history of xanthomatous involvement in other members of the family. Two (*Tel* and *Gef*) had suffered attacks of myocardial infarction, and only three (*Pol*, *Sen* and *Wol*) of the seven had been entirely free of symptomatic or electrocardiographic evidence of coronary heart disease. One of the patients (*Sen*), whose case will be described later in more detail, exhibited in addition to hypercholesterolemia a degree of hyperlipemia which brought into question the correctness of his inclusion in the group of primary hypercholesterolemic xanthomatosis. It could be contended that his condition should be classified as essential hyperlipemia.

Estinyl was given to six of these patients in the same dosage as was used for the survivors of myocardial infarction. Observations were made during periods when there were no perceptible variations in the clinical state. In the seventh patient (*Tel*), 2.0 mg. daily by mouth was substituted during a short period for the routine 1.0 mg. dose. Chronic cardiac decompensation with acute episodes of myocardial infarction, thrombophlebitis and pulmonary infarction complicated the period of experimental observation. The history of this patient has been published in detail elsewhere.[18] In *Sen*, lack of cooperation led to irregularity and to some uncertainty as to the exact dosage during the first period of administration.

Following the use of estinyl there was in the group but not in all its individuals a tendency toward reduction in the concentration of cholesterol in the unfractionated plasma and in Fraction I+III. As in the group of myocardial infarctions, the most constant change following the administration of estinyl was a shift in the distribution of cholesterol, with relative increase in Fraction IV+V+VI and corresponding decrease in Fraction I+III. In general the reduction in the concentration of phospholipids in Fraction I+III and in the unfractionated plasma was less than that of cholesterol, with resulting diminution of the cholesterol-phospholipid ratios. In Fraction IV+V+VI the change in concentration of cholesterol and phospholipids was usually proportional, so that the ratios varied little from pretreatment values.

In the case of *Tel*, judgment concerning the effects of estinyl was difficult because his clinical condition during the period of observation was constantly varied by the progress of his heart disease. Even in his case, however, the effects were perceptible. The percentage of cholesterol in the form of alpha lipoprotein was substantially increased, and that in the form of beta lipoprotein was correspondingly diminished. Cholesterol-phospholipid ratio in the plasma and in Fraction I+III was lower. It is noteworthy that in this case doubling of the usual daily dosage of estinyl for a period of seventeen days resulted in no augmentation of the chemical effects of the hormone.

Comment: The expected gonadal effects upon sexual desire and potency and upon the hyperplasia of the mammary glands were notable in all patients after a few weeks of treatment. In most cases, other clinical effects of the estinyl were confined to some gastric distress and a feeling of irritability or depression accompanying the impotence. Especially in patients with heart

failure, administration of estinyl resulted in retention of salt and water. In *Tel* this may have been a factor in the deterioration of his clinical condition, and it is possible that some of his attacks of pulmonary edema could be attributed to the action of the hormone.

with precise measurements before and after treatment failed to reveal any significant differences in size. In only one patient was it possible to demonstrate that estinyl had an effect on the xanthomatous deposits.

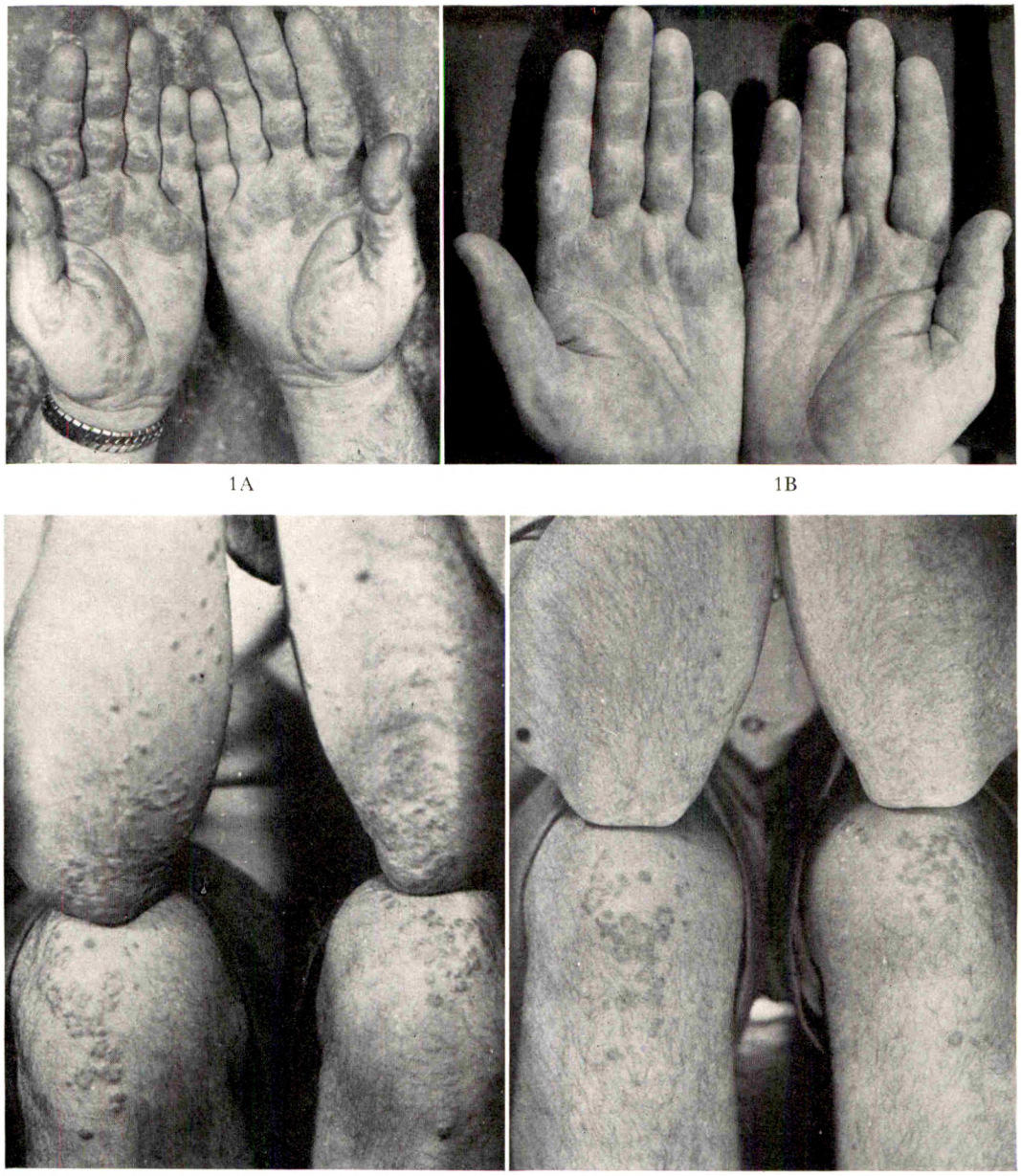
Sen was a skilled artisan who for several months

TABLE II

EFFECTS OF ADMINISTRATION AND WITHDRAWAL OF ESTINYL IN CASES OF PRIMARY HYPERCHOLESTEROLEMIC XANTHOMATOSIS

Subject, Sex and Age	Treatment		Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III		
						Cholesterol						
	Amount	Time (days)	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	(mg./100 ml.)	(% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
Tel M, 20	None	...	623	456	1.36	18	2.9	37	0.48	589	365	1.61
	Estinyl 1.0 mg.	19	649	557	1.16	34	5.4	71	0.47	589	441	1.33
	None	34	613	434	1.41	21	3.4	41	0.51	570	354	1.60
	Estinyl 1.0 mg.	27	711	640	1.11	38	5.5	57	0.66	644	543	1.17
	Estinyl 2.0 mg.	14	666	572	1.16	39	6.0	85	0.46	610	455	1.34
	None	19	715	544	1.31	22	3.1	49	0.44	662	472	1.40
Pol M, 26	None	...	356	...	....	28	7.8	...	....	326	...	....
	Estinyl 1.0 mg.	21	332	378	0.88	36	10.9	100	0.36	291	277	1.05
	Estinyl 1.0 mg.	40	302	334	0.90	47	15.7	114	0.41	248	215	1.15
	None	14	360	341	1.05	36	10.2	80	0.46	319	254	1.25
	None	21	340	322	1.05	33	10.1	70	0.47	296	246	1.20
Hid M, 27	None	...	506	...	....	49	9.7	...	....	456	...	....
	Estinyl 1.0 mg.	7	372	...	....	51	13.6	...	....	320	...	....
	Estinyl 1.0 mg.	26	384	376	1.02	67	17.4	153	0.44	315	223	1.41
	None	21	485	371	1.30	51	10.4	113	0.45	435	259	1.67
	None	45	457	327	1.39	46	10.1	69	0.66	400	244	1.63
Bie M, 30	None	...	494	516	0.96	29	5.9	73	0.39	449	425	1.05
	None	...	461	435	1.06	29	6.2	74	0.38	431	360	1.20
	Estinyl 1.0 mg.	21	358	337	1.06	67	19.1	123	0.55	285	211	1.35
	None	28	576	...	....	40	7.1	...	....	529	...	....
Sen M, 32	None	...	496	456	1.09	35	7.1	69	0.51	464	367	1.26
	Estinyl 1.0 mg.	16	...	...	....	..	....	...	....	...	...	....
	Estinyl 0.5 mg.	23	...	...	....	..	....	...	....	...	...	....
	None	10	311	...	....	40	12.7	...	....	274	...	....
	None	41	610	525	1.16	38	6.5	84	0.45	551	392	1.40
	None	96	626	500	1.25	39	6.3	77	0.51	579	425	1.36
	None	230	597	474	1.26	35	6.0	67	0.52	550	382	1.44
	Estinyl 1.0 mg.	10	424	399	1.06	43	10.0	88	0.49	383	304	1.26
	Estinyl 1.0 mg.	35	426	464	0.92	53	12.4	130	0.41	372	321	1.16
	Estinyl 1.0 mg.	77	487	530	0.92	55	11.4	139	0.39	426	362	1.18
	None	50	504	436	1.16	37	7.2	73	0.50	468	341	1.37
Gef M, 44	None	...	378	369	1.02	21	5.5	60	0.35	354	280	1.26
	Estinyl 1.0 mg.	22	342	315	1.08	38	11.6	75	0.51	288	218	1.32
	Estinyl 1.0 mg.	38	291	338	0.86	35	12.1	95	0.36	249	216	1.16
	Estinyl 1.0 mg.	50	281	...	....	41	14.6	...	....	241	...	....
	Estinyl 1.0 mg.	85	365	440	0.83	58	16.2	148	0.39	299	258	1.16
	Estinyl 1.0 mg.	108	340	416	0.82	46	13.6	124	0.37	290	265	1.10
	None	27	500	...	....	29	5.7	77	0.38	470	373	1.26
Wol F, 48	None	...	400	...	....	37	9.2	...	....	354	...	....
	None	...	424	...	....	35	8.5	...	....	369	...	....
	Estinyl 1.0 mg.	14	322	...	....	63	19.3	...	....	259	...	....
	Estinyl 1.0 mg.	28	347	...	....	58	17.2	...	....	279	...	....
	Estinyl 1.0 mg.	57	339	...	....	67	19.8	...	....	265	...	....
	None	14	413	...	....	52	13.4	...	....	328	...	....
	None	58	416	...	....	40	9.8	...	....	362	...	....

Observations were made on the effect of estinyl on the size and extent of xanthomatous lesions. In *Pol* and *Bie*, plaster casts of lesions had been unable to work because of painful xanthomas on his palms and the volar surfaces of his fingers. The deposits were accompanied



1A 1B

1C 1D

FIG. 1. Hands, elbows, and knees of patient *Sen* before (1A, 1C) and after (1B, 1D) treatment with estinyl.

by swelling, redness, heat and marked tenderness. He also had a few tuberous xanthomas on his elbows and knees. A single small lesion on an extensor tendon of the hand could be outlined. In some respects he differed from the other patients with xanthomatosis. The florid palmar lesions were in contrast to the sparsity of xanthomas elsewhere. There was no xanthelasma. It was noted also that his plasma was turbid, in contrast to other members of the group, and that the neutral fat of his plasma, with a value of 1,390 mg. per 100 ml., considerably exceeded the normal values obtained in our laboratory (71–335 mg. per 100 ml.).

During the first course of treatment the lesions on the hands, knees and elbows dramatically disappeared. Two weeks after he began to take estrogen the pain and swelling of his palms were improved sufficiently to permit him to resume his work. Because he was employed, some time

elapsed before he could return for examination. In the interval he suffered gastric distress which necessitated reduction in the dosage of estinyl from 1.0 to 0.5 mg. per day. He became intensely restive because of his impotence. It could not be ascertained whether he had taken the hormone regularly, but when he finally returned it was thought that he had taken 1.0 mg. of estinyl each day for thirty-eight days and 0.5 mg. daily for ten days.

Deposits in the palms, elbows and knees had disappeared, and their former locations were distinguishable only by discoloration of the skin. In Figure 1, the xanthomatous lesions of palms, elbows and knees may be seen before and after the administration of estinyl.

It is doubtful whether during his subsequent course the hormone was taken with any regularity. After three months of the prescription he was ordered to discontinue estinyl.

Return of xanthomatous lesions of the hands, elbows and knees was gradual, and nine months without treatment elapsed before the patient requested that the use of estinyl be resumed. Reinstitution of treatment was followed by prompt disappearance of the lipid deposits. The hormone was discontinued after two and one-half months. Six months later, the lesions were again present and troublesome.

It is remarkable that in this case there was such prompt disappearance of lesions, while in the others of the group the xanthomas were not obviously affected. The result is in accord with other observations that in idiopathic and acquired hyperlipemia lesions may be altered by diet and in appropriate circumstances by administration of insulin or thyroid.[19–23]

Although in *Sen*, as in other members of the group, estinyl did not accomplish complete restoration of a normal protein-lipid pattern, his clinical improvement suggests that in some instances partial chemical changes following use of the hormone may be an appropriate therapeutic goal.

C. Action of Oral Dosage of Premarin® (Estrone Sulfate) in Survivors of Myocardial Infarction. It was thought desirable to test a hormone closely resembling the natural estrogen, estrone; estrone sulfate, or premarin, was chosen for trial. It was calculated that 15 mg. of premarin contained approximately 10,000 rat units and could be regarded as approximately equivalent to 1.0 mg. of estinyl. Some of the experience with premarin in eight atherosclerotic patients is recorded in Table IIIA.

All of them had survived one or more myocardial infarctions, and two (*Noc* and *Yat*) had been subjects for observations with estinyl. *Bat* had been studied for many years as a case of myotonia congenita.

Various amounts of premarin, from 2.5 mg. to 22.5 mg. daily, were used over different periods of time. Five of the patients (*Gro*, *Lar*, *Pol*, *Kap* and *Bat*) received 15 mg. daily for significantly long periods. Because of the slight response of *Gro* to this amount, the dosage was increased to 22.5 mg. daily for a period of six weeks. In the other three cases, smaller amounts of the hormone were used.

From inspection of Table IIIA it can be seen that the action of premarin is qualitatively similar to that of estinyl. (Table I.) In the patients who received a supposedly equivalent amount of premarin, essential restoration to normal value was obtained in three (*Lar*, *Pol* and *Kap*). In *Gro*, who did not differ in any apparent way either clinically or chemically from other patients in the group, the effect of 15 mg. daily was less than had been expected. There was, however, even in this patient an increase in the relative amount of cholesterol in Fraction IV+V+V$_2$, a corresponding diminution in Fraction I+III, and a marked decrease in the cholesterol-phospholipid ratios of Fraction I+III and in the unfractionated plasma. The later use of a larger dose of 22.5 mg. did not augment the effect.

Slight chemical changes were observed in *Gue* with 7.5 mg. premarin daily; action was demonstrable in *Noc* with 5.0 mg., but was partially lost with a dose of 2.5 mg. daily. In *Yat*, who both before and after the use of premarin was shown to be very susceptible to the action of estinyl, no response could be shown from premarin in daily doses of 2.5 or 5 mg.

Comment: The observations on *Yat* with premarin indicate that at least one individual sensitive to estinyl was unresponsive to premarin in doses that in other patients (*Gue* and *Noc*) were sufficient to provide easily demonstrable chemical change. Symptomatically, the patients seemed to tolerate premarin better than estinyl. There were fewer complaints of gastric distress, nausea and general discomfort. The gonadal effects on the breasts, sexual desire and function

TABLE III

EFFECTS OF PREMARIN IN CASES OF MYOCARDIAL INFARCTION

Subject, Sex and Age	Date	State	Unfractionated Plasma			Fraction IV+V+V				Fraction I+III		
			Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Cholesterol (% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
A. Oral Administration												
Gro M, 48	2/11/52	No treatment	341	324	1.05	37	11.0	69	0.54	296	212	1.40
	5/29/52	15 mg. premarin for 15 wk.	*340*	*378*	*0.90*	*58*	*16.9*	*121*	*0.48*	*281*	*248*	*1.13*
	7/10/52	22.5 mg. premarin for 6 wk.	*316*	*360*	*0.88*	*44*	*14.0*	*132*	*0.33*	*270*	*226*	*1.19*
	7/24/52	No treatment for 2 wk.	376	397	0.95	39	10.3	...	....	338	257	1.31
Lar M, 48	4/29/52	No treatment	201	218	0.92	37	19.2	79	0.47	154	144	1.07
	7/24/52	15 mg. premarin for 10 wk	*180*	*271*	*0.66*	*66*	*36.7*	*150*	*0.44*	*115*	*94*	*1.22*
	8/7/52	No treatment for 2 wk.	210	293	0.72	69	33.0	162	0.43	136	107	1.27
Noc M, 43	4/17/52	No treatment	330	353	0.93	36	10.6	99	0.36	292	220	1.33
	5/1/52	No treatment	308	...	....	32	10.5	...	....	266	...	....
	7/1/52	5 mg. premarin for 9 wk.	*240*	*327*	*0.73*	*46*	*19.2*	*125*	*0.37*	*195*	*207*	*0.94*
	7/17/52	2.5 mg. premarin for 2 wk.	*276*	*354*	*0.78*	*45*	*16.1*	*123*	*0.37*	*233*	*233*	*1.00*
	9/18/52	No treatment for 9 wk.	276	319	0.87	34	12.1	86	0.40	246	227	1.08
Gue M, 42	4/24/52	No treatment for 10 wk.	318	336	0.95	69	21.4	126	0.54	249	187	1.33
	6/12/52	5 mg. premarin for 4 days	*308*	*367*	*0.84*	*66*	*21.4*	*137*	*0.48*	*234*	*174*	*1.34*
	6/27/52	7.5 mg. premarin for 15 days	*290*	...	....	*87*	*29.5*	...	....	*204*	...	....
Yat M, 49	8/7/53	No treatment for 6 wk.	347	370	0.94	38	11.4	115	0.33	290	216	1.34
	8/28/53	2.5 mg. premarin for 3 wk.	*330*	*344*	*0.96*	*34*	*10.7*	*94*	*0.36*	*283*	*223*	*1.27*
	10/23/53	5.0 mg. premarin for 8 wk.	*344*	*389*	*0.89*	*46*	*13.5*	*124*	*0.37*	*287*	*270*	*1.06*
Pol M, 52	2/13/52	No treatment	193	231	0.84	31	15.7	73	0.42	160	144	1.11
	6/5/52	15 mg. premarin for 16 wk.	*188*	*242*	*0.78*	*70*	*37.4*	*132*	*0.53*	*115*	*123*	*0.94*
	7/11/52	No treatment for 5 wk.	234	280	0.84	32	14.1	122	0.26	188	157	1.19
Kap M, 57	3/25/52	No treatment	231	241	0.96	30	13.5	68	0.44	192	156	1.23
	4/28/52	15 mg. premarin for 5 wk.	*220*	...	....	*50*	*23.2*	...	....	*161*	...	....
	5/15/52	No treatment for 1 wk.	194	268	0.72	43	22.5	96	0.45	146	148	0.99
Bat M, 51	3/31/52	No treatment	442	402	1.10	32	7.5	60	0.54	398	326	1.22
	5/8/52	15 mg. premarin for 5 wk.	*336*	*391*	*0.86*	*44*	*12.9*	*98*	*0.44*	*294*	*280*	*1.05*
	5/22/52	No treatment for 2 wk.	390	373	1.05	25	6.4	63	0.40	363	300	1.21
B. Single Intravenous Dose												
Noc M, 43	6/9/53	No treatment for 19 days	282	322	0.88	37	13.0	85	0.43	242	206	1.17
	6/9/53	Premarin 20 mg. intravenously	...	...	....	..	....	...	....	...	...	....
	6/10/53	Blood drawn 24 hr. after injection of premarin	254	280	0.91	28	10.9	75	0.38	230	199	1.15
	6/16/53	No treatment for 7 days	293	308	0.95	38	13.3	90	0.43	249	209	1.19
	7/1/53	No treatment for 20 days	275	266	1.03	30	11.8	88	0.43	244	197	1.24
	7/1/53	Premarin 20 mg. intravenously	...	...	....	..	....	...	....	...	...	....
	7/2/53	Blood drawn 24 hr. after injection of premarin	290	278	1.04	42	13.9	80	0.52	259	197	1.31
	7/8/53	No treatment for 7 days	290	285	1.02	35	11.9	81	0.43	256	205	1.25
Yat M, 49	6/10/53	No treatment for 40 days	354	289	1.23	44	12.5	84	0.53	307	194	1.58
	6/10/53	Premarin 15 mg. intravenously	...	...	....	..	....	..	....	...	...	....
	6/11/53	Blood drawn 24 hr. after injection of premarin	322	294	1.06	41	12.9	88	0.46	269	194	1.41
	6/23/53	No treatment for 3 days	338	306	1.10	40	11.8	96	0.42	294	209	1.41
	7/1/53	No treatment for 20 days	336	293	1.15	37	11.0	87	0.49	292	210	1.39
	7/1/53	Premarin 20 mg. intravenously	...	...	....	..	....	...	....	...	...	....
	7/2/53	Blood drawn 24 hr. after injection of premarin	372	308	1.21	45	12.0	88	0.53	324	224	1.45
	7/7/53	No treatment for 7 days	375	300	1.25	43	11.7	87	0.54	320	223	1.43
Mra M, 38	6/30/53	No treatment	306	282	1.05	37	12.0	83	0.45	269	199	1.35
	6/30/53	Premarin 20 mg. intravenously	...	...	....	..	....	...	....	...	...	....
	7/1/53	Blood drawn 24 hr. after injection of premarin	290	276	1.05	39	13.4	78	0.51	248	189	1.31
	7/6/53	No treatment for 6 days	332	292	1.12	47	14.6	92	0.51	269	200	1.35

Note: Figures in italics indicate results on days of treatment by oral administration.

were indistinguishable when equivalent doses were given.

Partial confirmation of our observations with premarin have been furnished by Marett and Vivas[24] who observed in twelve survivors of myocardial infarction significant reduction in concentration of cholesterol and total lipid following the oral administration of premarin in doses of 5 mg. (1.25 mg. four times a day). In their series, similar effects from premarin were evident in one postmenopausal woman, in two cases of mild hypertension without clinical evidence of coronary atherosclerosis, in two cases diagnosed as atherosclerosis without myocardial infarction, and in one patient with severe generalized pruritus who presented no stigmata of vascular disease.

D. Action of Intravenous Doses of Premarin (Estrone Sulfate). In three survivors of myocardial infarction, study was made of the action of a single intravenous dose of premarin. These experiments were prompted by a report of Gitman and Greenblatt[25] that by means of this procedure they were able to diminish the concentration of beta lipoproteins of the S_f 12–20 and 20–100 classes in the serum of a patient with coronary insufficiency.

Our results are shown in Table IIIB. Two of the subjects (*Noc* and *Yat*) had been treated previously with estinyl and premarin. The other patient (*Mra*) had not received any estrogen therapy. No significant changes were seen to follow injection in doses exceeding the amounts used by Gitman and Greenblatt.

Comment: It is obvious that premarin given intravenously in single doses of 15 to 20 mg. exerted no effect on the concentration or distribution of the alpha and beta lipoproteins as observed by our chemical methods of separation. It should be mentioned, however, that the criteria in our experiments and in those of Gitman and Greenblatt are quite different, and that our experiences cannot be regarded as necessarily contradictory.

E. Action of Diethylstilbestrol. The estrogen, diethylstilbestrol, was tested in three survivors of myocardial infarction for a period of one week. Starting with 1.0 mg. during the first twenty-four hours and raising the dose by 1.0 mg. each day, the total amount for eight days was 36 mg. Calculated on the basis that 1.0 mg. diethylstilbestrol is equal to 1,200 rat units, this dosage represented approximately 43,200 rat units a week, or the equivalent of approximately one-half the weekly amount used in the estinyl experiments (10,000 rat units daily). The results are shown in Table IVA.

Two of the cases (*Yat* and *Noc*) had been studied in previous experiments for the effects of the use of both estinyl and oral premarin. The third patient (*Mra*) had received intravenous premarin but had not been tested with other oral preparations.

With diethylstilbestrol, the chemical changes observed in two of the three patients (*Noc* and *Mra*) were similar to those seen following the use of estinyl and premarin. There was a decrease in the concentration of cholesterol in the plasma and in Fraction I+III, with a corresponding decrease in the cholesterol-phospholipid ratios, and an increase in the concentration and distribution of lipids in Fraction IV+V+VI, with no alteration in the cholesterol-phospholipid ratios. In these two patients, the changes were relatively large and were reversed immediately following the discontinuance of the hormone. In the case of *Yat*, the patient who had responded poorly to small doses of premarin, the influence of diethylstilbestrol was barely perceptible.

Comment: Observations with diethylstilbestrol are fewer and were carried out over a shorter period of time. They are therefore not strictly comparable to those following use of estinyl and premarin. It is apparent, however, that diethylstilbestrol produces similar changes in the protein-lipid relationships of plasma.

F. Action of Estradiol Benzoate. The effects of the oral administration of estradiol benzoate in daily doses of 1.66 mg. were studied in a male patient who was convalescent from spinal fusion in a case of tuberculous spondylitis. It was not apparent that he had any stigmata or complications of atherosclerosis.

Estrogen treatment was instituted about six weeks after the operation was performed. Throughout the course, he was treated with streptomycin, paraminosalicylic acid and nitrozide. He was entirely afebrile and asymptomatic during the period of chemical observation.

From Table IVB it may be seen that the effects of estradiol benzoate on the protein-lipid patterns were qualitatively similar to the other estrogens. The concentrations of cholesterol in the plasma and in Fraction I+III were decreased, and the percentage of cholesterol in Fraction IV+V+VI was increased. The cholesterol-phospholipid ratio of the unfractionated

TABLE IV

EFFECTS OF VARIOUS ESTROGENS IN A VARIETY OF CONDITIONS

Subject, Sex and Age	Date	State	Protein				Lipids									
			Total Protein (Kjeldahl) (gm./100 ml.)	Protein in Fractions (Biuret) (gm./100 ml.)			Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III		
				IV+V+VI	II	I+III	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	(% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
A. Effects of Administration and Withdrawal of Diethylstilbestrol																
Noc M, 43	10/1/53	No treatment for 3 mo.					320	300	1.11	33	10.4	79	0.42	288	211	1.37
	10/1/53	Diethylstilbestrol started					...	...	....	..	....	...	....	....	...	....
	10/8/53	Diethylstilbestrol discontinued after total dosage of 36 mg.					290	318	0.91	45	15.4	106	0.43	246	212	1.16
	10/14/53	No treatment for 6 days					336	357	0.94	48	14.5	100	0.49	280	248	1.13
	10/29/53	No treatment for 21 days					334	284	1.18	33	10.1	72	0.46	292	218	1.34
Yat M, 49	10/1/53	No treatment for 3 mo.					362	312	1.19	38	10.8	86	0.44	315	213	1.48
	10/1/53	Diethylstilbestrol started					...	...	...	..	....	...	....	...	...	....
	10/8/53	After total dosage of 36 mg.					333	333	1.00	48	13.6	97	0.49	299	224	1.33
	10/10/53	Diethylstilbestrol discontinued					324	287	1.13	43	13.5	86	0.50	272	186	1.47
	10/15/53	No treatment for 5 days					366	306	1.20	48	13.4	89	0.54	306	202	1.51
	11/5/53	No treatment for 25 days					...	...	....	..	....	...	....	...	...	....
Mra M, 38	10/7/53	No treatment for 3 mo.					274	211	1.30	37	13.9	62	0.60	226	145	1.56
	10/7/53	Diethylstilbestrol started					...	...	...	...	....	...	....	...	...	....
	10/14/53	Diethylstilbestrol discontinued after total dosage of 36 mg.					247	283	0.87	48	19.7	100	0.48	192	183	1.05
	10/20/53	No treatment for 6 days					299	300	1.00	56	15.5	70	0.80	304	212	1.43
	11/11/53	No treatment for 22 days					376	320	1.18	46	12.7	81	0.57	309	220	1.41
B. Effects of Administration of Estradiol Benzoate in Case of Tuberculous Spondylitis Convalescent Following Spinal Fusion																
Vau M, 23	2/10/53	No treatment					160	203	0.79	33	20.9	67.9	.49	120	114	1.05
	2/25/53	Estradiol benzoate 1.66 mg. o.d. started					133	160	0.83	38	29.4	75.0	.51	88	94	0.94
	3/11/53						116	153	0.76	30	25.4	58.3	.51	85	78	1.07
	3/30/53						126	175	0.72	35	28.5	78.7	.45	86	83	1.04
	4/14/53						135	175	0.77	37	28.4	79.0	.47	91	84	1.08
	5/5/53						115	170	0.68	36	31.0	81.4	.44	78	75	1.04
C. Effects of Estrogens in the Nephrotic Syndrome																
Wie M, 19	5/17/52	No treatment	3.5	...	...	...	805	649	1.25	..	....	...	....	671	506	1.33
	6/2/52	Premarin 15 mg. o.d. started	3.6	1.2	0.1	2.6	667	616	1.08	17	2.5	45	0.38	653	536	1.22
	6/12/52		3.7	1.1	0.1	2.6	622	561	1.11	19	3.0	44	0.45	605	488	1.24
	7/1/52	Premarin discontinued	3.7	1.4	0.2	2.4	702	708	0.99	24	3.4	58	0.42	676	596	1.13
			5.2	2.8	0.3	2.1	467	340	1.37	56	11.9	105	0.53	407	235	1.73
Has M, 22	3/18/54	No treatment	5.2	3.3	0.4	1.7	410	324	1.26	56	13.8	86	0.65	352	231	1.52
	4/12/54	Estinyl 1.0 mg. o.d. started														
	4/19/54		4.7	2.5	0.3	1.9	385	325	1.20	63	16.7	98	0.64	310	206	1.50
	4/26/54		4.3	2.2	0.3	2.0	402	356	1.13	58	14.3	99	0.58	345	241	1.43
	5/3/54		4.1	2.1	0.2	1.9	464	376	1.21	63	14.1	100	0.63	378	251	1.40
			3.9	1.9	0.3	1.8	428	373	1.15	53	12.2	90	0.59	378	264	1.43
	5/11/54	Estinyl discontinued														
	5/17/54		4.3	2.2	0.3	1.8	562	420	1.34	54	9.9	93	0.59	489	302	1.62
	5/24/54		...	2.2	0.2	1.7	596	440	1.35	50	8.3	90	0.55	543	316	1.72

plasma was diminished after approximately two and one-half months.

Comment: This experiment with the use of estradiol benzoate demonstrates the action of another estrogen as capable of modifying the protein-lipid relationships in plasma and offers further evidence that the effects of estrogens are not limited to survivors of myocardial infarction.

G. Action of Estrogens in the Nephrotic Syndrome. Data obtained following the use of estrogens in two patients with the nephrotic syndrome are assembled in Table IVC.

In the first patient (*Wie*) acute nephritis developed at the age of eleven. At the time of observation he had massive edema and 3+ albuminuria with slight hematuria. The blood urea nitrogen concentration was 31 mg. per 100 ml., with a urea clearance 35 per cent of normal. Separation of the lipoproteins was unsatisfactory at this time because of the difficulties which are occasionally encountered during the fractionation of nephrotic plasmas.[14] This did not, however, affect the analyses for protein and lipid concentrations of the unfractionated plasma, the values of which are listed in Table IVC, together with ratios of cholesterol to phospholipid. He received premarin in daily doses of 15 mg. for six weeks. Chemically, there was an abrupt fall in the concentration of plasma cholesterol, and the cholesterol-phospholipid ratio was progressively diminished. Protein fractionations which were satisfactory throughout this period showed little change except for a tendency toward an increase in the cholesterol concentration in Fraction IV+V+VI. Clinically, he was little affected by the treatment.

In the second patient (*Has*) acute nephritis developed at the age of fourteen and thereafter the patient continued to have albuminuria and hypertension. In January, 1954, when first examined at The New York Hospital, he was found to have a blood pressure of 170/110, with no edema. The urine contained 4+ albumin, red blood cells and casts. The concentration of blood urea nitrogen was 10 mg. per 100 mL, and the serum protein was 4.9 gm. per 100 mL, with 2.9 gm. of albumin and 2.0 gm. of globulin. The urea clearance was 68 per cent of normal.

At the time of his first plasma fractionation, estinyl (1.0 mg. o.d.) with limitation of salt intake was prescribed. Inspection of Table IVC shows that in this case the concentration of cholesterol tended to be somewhat lower, and the percentage of cholesterol in Fraction IV+V+VI slightly higher during the administration of the hormone. The cholesterol-phospholipid ratios, always high, were somewhat reduced. Discontinuance of estinyl resulted in prompt return to values more abnormal than those originally observed. Qualitatively, the changes were like those observed with the use of estinyl in the groups of survivors of myocardial infarction and in patients with primary hypercholesterolemic xanthomatosis. Quantitatively, however, they were so small that they could not be regarded as significant except in the light of previous experience in the other groups.

Much more evident were the changes in the protein values. Following the administration of estinyl there was a large reduction in the concentration of total protein which was almost entirely accounted for by a fall in the concentration of protein (presumably albumin) in Fraction IV+V+VI. After discontinuance of the hormone there was a restoration of the protein to values approximating those observed before treatment was begun. Such effects on protein concentration were not seen in any of the nonnephrotic patients following the use of estinyl.

Comment: Symptomatically, the second patient was disturbed by the medication. During the first two days he complained of nausea and experienced unusual fatigue. He vomited on one occasion and complained that he was constantly uncomfortable. His breasts became sore after he had taken the hormone for about three weeks. Since discomfort and unfavorable chemical changes were noted in the second patient and little if any chemical changes were observed in the first patient, additional experiments with estrogens in other cases of nephrosis were discouraged.

II. Effects of Androgens

A study of the effects of androgens was initiated soon after we had demonstrated sex difference in the distribution of alpha and beta lipoproteins.[1]

A. Action of Oral Administration of Methyl Testosterone. In the preliminary study of androgen effects, methyl testosterone was selected for trial not only because of the convenience of an oral preparation but also because it had been so extensively used for clinical purposes. It was realized that it might not reflect accurately the action of other forms of testosterone. It was recognized also that some adverse effects on the liver had been reported.[26]

TABLE V

EFFECTS OF ADMINISTRATION AND WITHDRAWAL OF METHYL TESTOSTERONE (M.T.) IN A VARIETY OF CONDITIONS

Subject, Sex and Age	Diagnosis	Date	State	Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III		
				Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Cholesterol (% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
Bar M, 64	Normal	7/25/52	No treatment	212	...	....	60	28.4	...	....	152	...	....
		8/12/52	M.T. 50 mg. o.d. for 10 days	*209*	*285*	*0.73*	*37*	*18.3*	*110*	*0.35*	*168*	*167*	*1.01*
Rus F, 36	Normal	6/4/52	No treatment	157	...	....	64	40.9	...	....	93	...	....
		6/4/52	M.T. 10 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
		6/22/52	M.T. discontinued after total dose of 130 mg.	...	...	....	..	....	...	....	...	...	....
		6/23/52	No treatment for 24 hr.	145	...	....	38	26.4	...	....	102	...	....
		7/15/52	No treatment for 10 days	140	...	....	45	32.0	...	....	93	...	....
Lar M, 48	Survivor infarction	11/3/52	No treatment	186	227	0.82	55	30.2	118	0.46	121	96	1.21
		11/25/52	M.T. 50 mg. o.d. for 10 days	*169*	*175*	*0.97*	*27*	*16.4*	*67*	*0.40*	*137*	*108*	*1.27*
		2/13/52	No treatment for 11 wk.	182	248	0.73	52	29.0	118	0.44	124	110	1.13
Spi M, 29	Survivor infarction	10/27/55	No treatment	253	245	1.03	58	22.6	118	0.49	199	144	1.39
		11/20/52	M.T. 5 mg. o.d. for 24 days	*236*	*203*	*0.98*	*32*	*13.6*	*72*	*0.45*	*205*	*130*	*1.57*
		12/19/52	No treatment for 29 days	201	231	0.87	43	20.2	102	0.42	161	124	1.29
Cud M, 20	Convalescent poliomyelitis	9/22/52	No treatment	169	174	0.97	35	21.5	67	0.52	128	103	1.24
		10/8/52	M.T. total dose of 450 mg. in 16 days	*167*	*176*	*0.96*	*20*	*12.1*	*57*	*0.35*	*146*	*126*	*1.20*
		12/15/52	No treatment for 66 days	162	186	0.87	40	24.9	86	0.46	117	96	1.22
Lan M, 35	Convalescent poliomyelitis	9/16/52	No treatment	195	233	0.84	55	27.9	104	0.53	139	118	1.19
		9/30/52	M.T. total dose of 400 mg. in 15 days	*177*	*170*	*1.05*	*39*	*21.6*	*77*	*0.50*	*140*	*107*	*1.30*
		10/22/52	No treatment for 20 days	203	234	0.87	48	24.0	105	0.45	147	120	1.22
Wai M, 20	Convalescent poliomyelitis	9/16/52	No treatment	180	196	0.92	47	26.1	93	0.51	134	99	1.35
		9/30/52	M.T. total dose of 400 mg. in 15 days	*165*	*194*	*0.94*	*26*	*15.9*	*57*	*0.45*	*135*	*120*	*1.13*
		10/15/52	No treatment for 13 days	204	202	1.01	45	22.0	78	0.58	160	111	1.40
Had M, 26	Convalescent poliomyelitis	9/16/52	No treatment	177	171	1.04	29	16.7	66	0.43	138	118	1.17
		9/30/52	M.T. total dose of 400 mg. in 15 days	*157*	*188*	*0.84*	*21*	*13.5*	*56*	*0.36*	*132*	*130*	*1.02*
		10/8/52	No treatment for 6 days	185	200	0.93	24	13.3	64	0.38	159	135	1.19
Jan F, 16	Afebrile tuberculosis	8/11/52	No treatment	195	...	....	30	16.0	...	....	156	...	....
		9/2/52	M.T. total dose of 850 mg. in 19 days	*163*	*212*	*0.77*	*22*	*13.1*	*61*	*0.35*	*143*	*130*	*1.10*
		9/18/52	No treatment for 16 days	148	178	0.83	32	21.5	68	0.47	117	109	1.07
Cra F, 57	Afebrile tuberculosis	8/1/52	No treatment	200	...	....	35	18.1	...	....	152	...	....
		8/18/52	M.T. 50 mg. o.d. for 17 days	*256*	*266*	*0.96*	*22*	*8.7*	*60*	*0.36*	*232*	*193*	*1.20*
		9/23/52	No treatment for 37 days	232	252	0.92	39	17.4	88	0.44	181	154	1.19
Rap F, 20	Afebrile tuberculosis	8/13/52	No treatment	218	241	0.91	40	18.9	99	0.40	169	120	1.39
		8/27/52	M.T. 50 mg. o.d. for 14 days	*188*	...	....	*25*	*13.6*	...	....	*157*	...	....
		9/9/52	No treatment for 13 days	187	204	0.92	45	23.8	88	0.51	144	118	1.21

TABLE V (*Continued*)

Subject, Sex and Age	Diagnosis	Date	State	Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III		
				Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Cholesterol (% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
Gum F, 22	Afebrile tuberculosis	8/22/52	No treatment	180	269	0.67	50	27.6	120	0.41	130	126	1.03
		9/9/52	M.T. 50 mg. o.d. for 14 days	*250*	*268*	*0.93*	*33*	*13.3*	*72*	*0.46*	*214*	*177*	*1.21*
		9/22/52	No treatment for 14 days	196	245	0.80	40	20.4	88	0.45	157	151	1.04
Bil F, 41	Endometriosis	5/2/52	No treatment	188	238	0.79	49	27.1	98	0.49	126	109	1.16
		6/26/52	T.P. 25 mg. intramuscularly, o.d. except Sat. and Sun.	*212*	*201*	*1.05*	*34*	*16.3*	*68*	*0.52*	*175*	*133*	*1.32*
			M.T. 100 mg. p.o. Sat. and Sun. for 8 wk.	...	...	....	...	....	...	....	...	...	....
		7/15/52	No treatment for 19 days	190	230	0.83	49	26.4	99	0.49	136	104	1.30
Maj F, 37	Endometriosis	6/24/52	No treatment	210	242	0.87	38	18.0	87	0.45	165	150	1.10
		7/22/52	M.T. 100 mg. o.d. for 26 days	*271*	*222*	*0.96*	*22*	*10.4*	*57*	*0.39*	*191*	*149*	*1.28*
		8/14/52	No treatment for 25 days	208	219	0.95	34	16.7	74	0.47	172	133	1.29
Haa F, 39	Endometriosis	10/14/52	No treatment	152	207	0.73	41	26.7	101	0.41	113	99	1.15
		11/11/52	M.T. 100 mg. o.d. for 22 days	*392*	*339*	*1.15*	*28*	*17.3*	*60*	*0.46*	*351*	*250*	*1.40*
			M.T. 50 mg. o.d. for 4 days	...	...	....	..	....	...	....	...	...	....
		12/16/52	No treatment for 36 days	172	201	0.86	47	28.1	97	0.49	119	108	1.01
Ruo M, 64	Aortic valvular heart disease	8/25/52	No treatment	185	271	0.68	31	16.9	71	0.44	152	136	1.11
		9/8/52	M.T. 50 mg. o.d. for 14 days	*248*	*288*	*0.86*	*24*	*10.3*	*71*	*0.34*	*209*	*207*	*1.00*
Biz F, 48	Menopause	12/1/50	No treatment	288	...	....	79	29.9	...	....	177	...	...
		12/19/50	T.P. 25 mg. intramuscularly, o.d. except Sat. and Sun.	*249*	...	....	*51*	*22.2*	...	....	*169*	...	....
			M.T. 100 mg. p.o. Sat. and Sun. for 18 days	...	...	....	..	....	...	....	...	...	....
Rob F, 55	Menopause	11/22/50	No treatment	283	...	....	48	18.4	...	....	210	...	....
		12/13/50	T.P. 25 mg. intramuscularly o.d. except Sat. and Sun.	*376*	...	....	*32*	*11.1*	...	....	*252*	....	....
			M.T. 100 mg. p.o. Sat. and Sun. for 18 days	...	...	....	..	....	...	....	...	...	....
Hoo M, 29	Hypogonadism	10/21/52	Testosterone implant 5 mo. previously	211	296	1.05	40	13.2	81	0.49	259	205	1.24
		12/2/52	M.T. 50 mg. o.d. for 41 days	*484*	*394*	*1.23*	*19*	*4.1*	*43*	*0.44*	*445*	*350*	*1.27*
		12/18/52	No treatment for 17 days	346	342	1.01	32	9.4	73	0.44	302	262	1.15
Dal M, 50	Hypogonadism	11/3/52	Testosterone implant 2 mo. previously	248	265	0.94	47	19.2	99	0.47	192	160	1.20
		11/11/52	M.T. 50 mg. o.d. for 7 days	*239*	*249*	*0.96*	*31*	*13.1*	*76*	*0.41*	*208*	*176*	*1.18*
		12/23/52	No treatment for 43 days	261	299	0.87	44	16.9	96	0.46	212	193	1.10
Rud M, 29	Hypogonadism	10/20/52	No treatment	190	220	0.86	45	23.7	104	0.43	139	106	1.31
		11/13/52	M.T. 50 mg., o.d. for 17 days	*207*	*175*	*1.18*	*23*	*11.4*	*54*	*0.43*	*181*	*121*	*1.46*

Note: Figures in italics represent results on days of treatment.

Routinely, methyl testosterone was given in daily doses of 50 mg. As with the estrogens, the choice of dose was arbitrary and was made with the hope that the amount would be sufficiently large to exert easily demonstrable chemical changes without toxic action. The dosage was varied only occasionally during the course of treatment. Random observations were made on the combined effects of testosterone propionate and methyl testosterone in three endocrine patients.

Some of the changes observed following the use of methyl testosterone have been assembled in Table v. The group of subjects included two normal individuals as well as seventeen patients with various disease states and two women with symptoms of the menopause.

The results may be simply stated. In every instance there was a change in the distribution of cholesterol in a direction opposite to that observed following the administration of estrogen. Both relative and absolute amounts of cholesterol and phospholipids in Fraction IV+V+VI were decreased, and those in Fraction I+III were correspondingly increased. In ten of the twenty-one subjects the concentration of

TABLE VI

EFFECTS OF SIMULTANEOUS ADMINISTRATION OF ESTINYL AND METHYL TESTOSTERONE

Subject, Sex and Age	Date	State	Unfractionated Plasma			Fraction IV+V+VI				Fraction I+III		
						Cholesterol						
			Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio	(mg./100 ml.)	(% of total)	Phospholipids (mg./100 ml.)	C/Pl Ratio	Cholesterol (mg./100 ml.)	Phospholipids (mg./100 ml.)	C/Pl Ratio
Gil M, 39	9/8/52	No treatment for 42 days	317	374	0.85	30	9.4	85	0.36	290	281	1.03
	9/8/52	M.T. 50 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
	9/22/52	Estinyl 1.0 mg. o.d. started	319	341	0.94	22	6.9	52	0.41	290	280	1.04
	10/16/52	M.T. discontinued	368	...	....	13	3.6	...	....	360	...	....
	11/6/52	Estinyl dosage reduced to 0.25 mg. o.d.	269	332	0.81	56	21.7	139	0.41	202	192	1.05
	12/18/52	Estinyl discontinued	217	315	0.69	68	31.1	158	0.43	147	149	0.99
	2/5/53	No treatment for 48 days	314	325	0.97	31	9.8	77	0.40	281	269	1.04
Noe M, 43	9/18/52	No treatment for 62 days	276	319	0.87	34	12.1	86	0.40	246	227	1.08
	9/18/52	M.T. 50 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
	10/9/52	Estinyl 1.0 mg. o.d. started	318	363	0.88	13	4.1	51	0.25	291	304	0.96
	10/23/52	M.T. discontinued	366	405	0.91	19	5.3	69	0.27	338	372	0.91
	10/30/52	Estinyl dosage reduced to 0.5 mg. o.d.	304	299	1.02	28	9.1	72	0.39	276	242	1.10
	11/11/52	Estinyl discontinued	241	323	0.75	49	21.0	124	0.39	183	194	0.94
Yat M, 49	8/7/52	No treatment for 42 days	347	370	0.94	38	11.4	115	0.33	290	216	1.34
	8/7/52	Premarin 0.25 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
	8/28/52	Premarin dosage increased to 0.5 mg. o.d.	330	344	0.96	34	10.7	94	0.36	283	223	1.27
	10/23/52	Premarin discontinued	344	389	0.89	46	13.5	124	0.37	287	270	1.06
		Estinyl 1.0 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
	1/15/53	M.T. 50 mg. o.d. started	195	290	0.67	54	28.7	134	0.40	134	147	0.91
	2/11/53	M.T. discontinued	289	363	0.80	14	4.7	56	0.24	276	313	0.88
	4/2/53	Estinyl discontinued	186	280	0.67	46	24.8	125	0.37	139	152	0.91
	4/20/53	No treatment for 18 days	242	312	0.78	45	19.0	123	0.36	190	185	1.03
Gre M, 53	1/17/52	No treatment	202	...	....	19	9.7	...	....	171	...	....
	1/17/52	Estinyl 1.0 mg. o.d. started	...	...	....	..	....	...	....	...	...	....
	5/22/52	Estinyl dosage reduced to 0.5 mg. o.d.	173	281	0.62	50	29.6	137	0.37	120	117	1.03
	8/1/52	Estinyl dosage reduced to 0.25 mg. o.d.	194	275	0.71	66	33.1	...	....	...	...	....
	9/25/52	M.T. 50 mg. o.d. started	217	296	0.73	68	31.2	155	0.44	148	138	1.07
	10/23/52	M.T. discontinued	351	362	0.97	11	3.2	40	0.28	338	308	1.10
	12/11/52	M.T. 10 mg. o.d. started	226	278	0.81	62	27.6	146	0.42	161	140	1.14
	2/5/53	Estinyl dosage increased to 1.0 mg. o.d.	274	254	1.08	21	8.0	63	0.34	247	190	1.30
	3/12/53	Estinyl dosage reduced to 0.5 mg. o.d.	261	276	0.95	21	8.3	61	0.35	231	198	1.17
	4/9/53	M.T. dosage reduced to 5.0 mg. o.d.	248	268	0.93	19	8.0	55	0.35	222	202	1.10
	5/14/53	All hormones discontinued	221	212	1.04	32	14.5	71	0.45	187	137	1.36

cholesterol in the unfractionated plasma was augmented, and in two of them (*Haa* and *Hoo*) the increase was very great. In the remaining eleven subjects, the cholesterol concentration was slightly diminished. Nine of the patients displayed a sharp rise in the cholesterol-phospholipid ratios of plasma and of Fraction I+III following treatment. In several, there was a tendency toward a decreased ratio in Fraction IV+V+VI.

Limited observations of the three cases (*Bil*, *Rob* and *Biz*) in which testosterone propionate formed the major part of the regimen are also shown in Table V. It may be significant that the changes were in the same direction and somewhat greater than the average change observed when methyl testosterone was given alone.

Cholesterol partitions were performed on the plasma of ten patients who received testosterone. In every instance the free to total ratio was increased following the administration of the hormone. The average before treatment of 0.26 with variation between 0.23 and 0.30, may be compared with 0.33, with variation of 0.27 to 0.39 after treatment.

Comment: The action of methyl testosterone produced in normal individuals and in a variety of patients who exhibited no stigmata of circulatory disease, protein-lipid patterns that approached those found in survivors of infarction. The abnormalities in protein-lipid relationships in the plasma of survivors of myocardial infarction were even more exaggerated following the use of this hormone. No hepatic disease was noted during the course of these experiments.

III. Effects of Simultaneous Administration of Estrogens and Androgens

Since estrogens and androgens were found to produce opposite effects on the concentration and distribution of lipids in the lipoproteins of plasma, studies were undertaken to ascertain how the plasma would be affected by simultaneous administration of the two oppositely acting substances. Some of the results of four such experiments on survivors of myocardial infarction are graphically depicted in Figure 2. A more detailed summary of the data may be seen in Table VI. All of the subjects had served in previous experiments on the effects of estrogen, and for comparative purposes some of the results of these experiments are included.

In both *Gil* and *Noc* the administration of methyl testosterone exaggerated the abnormalities of their untreated state by increasing the cholesterol concentration and cholesterol-phospholipid ratio of the unfractionated plasma and by diminishing the percentage of cholesterol in Fraction IV+V+VI. Although both patients had previously responded promptly to the action of estrogens, the simultaneous administration of 1.0 mg. estinyl had no effect in modifying the action of 50 mg. of methyl testosterone. There was no reversal of the lipid values toward pretreatment levels. Upon the withdrawal of methyl testosterone and the continuation of estrogen in even smaller doses (0.25 mg. and 0.5 mg. o.d.), there was a partial re-establishment of a normal pattern. The plasma cholesterol concentrations and the cholesterol-phospholipid ratios were decreased to approximately normal ranges; and there was a marked increase in both the amount of distribution of the lipids in Fraction IV+V+VI, with a corresponding decrease in Fraction I+III. Following the withdrawal of methyl testosterone, *Gil* maintained a normal pattern for nearly six weeks on only 0.25 mg. estinyl daily.

In *Yat* and *Gre* the plan of experimentation was altered. Methyl testosterone in daily doses of 50 mg. was added without interrupting the administration of estinyl, which in the case of *Yat* was given in daily doses of 1.0 mg. for three months and in *Gre* in doses reduced from 1.0 mg. to 0.25 mg. over a period of seven months. The powerful action of methyl testosterone in counteracting the effects of estinyl is shown in *Gre*. The concentration of cholesterol was increased 134 mg. per 100 ml. and that of phospholipids 66 mg. per 100 ml. The percentage of cholesterol in Fraction IV+V+VI dropped from 31.2 to 3.2, a value which was far below the pretreatment level. Both cholesterol and phospholipid concentrations were diminished in Fraction IV+V+VI with a corresponding increase in Fraction I+III. At the same time, the plasma cholesterol-phospholipid ratio increased from 0.73 to 0.97.

When methyl testosterone was discontinued, the effects of 0.25 mg. estinyl were again evident, for the plasma cholesterol concentration was decreased by 125 mg. per 100 ml. accompanied by a fall in the cholesterol-phospholipid ratio from 0.97 to 0.81. Both cholesterol and phospholipid concentrations in Fraction IV+V+VI were markedly increased, while those in Fraction I+III were correspondingly diminished.

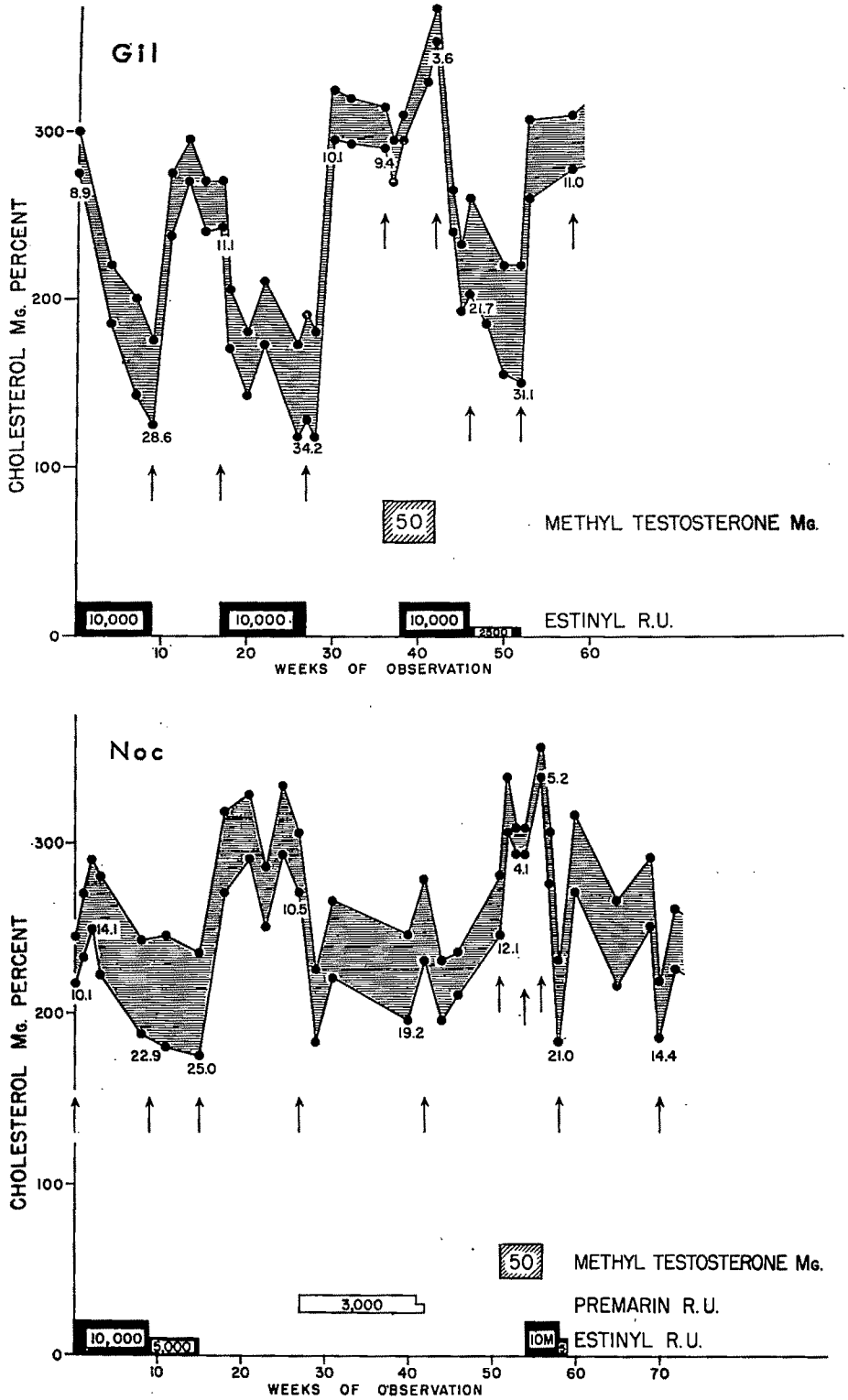


FIG. 2A. Patients *Gil* and *Noc*. Effects of estrogens and androgens in survivors of myocardial infarction (for explanation of chart construction, see legend on page 21).

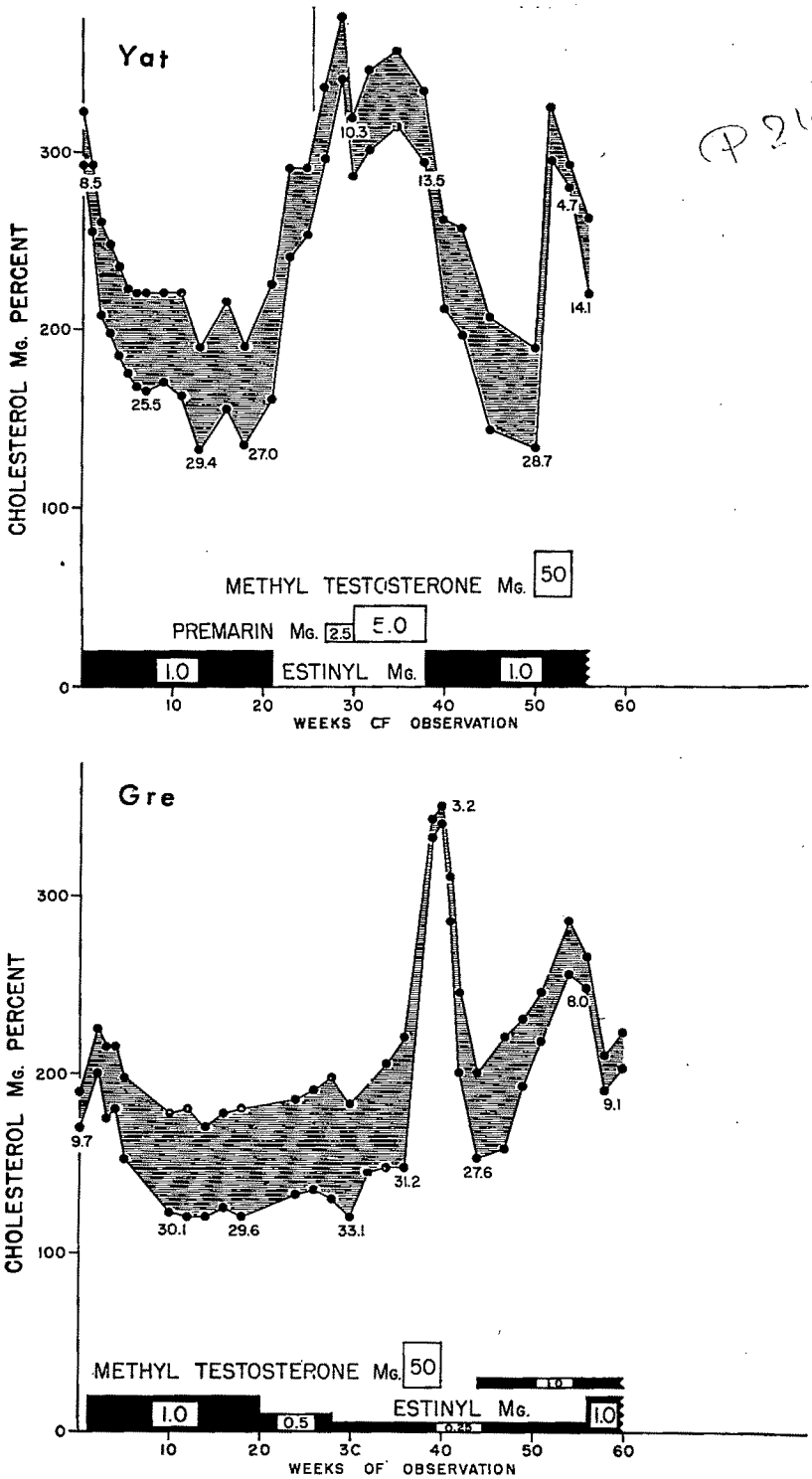


FIG. 2B. Patients *Yat* and *Gre*. Effects of estrogens and androgens in survivors of myocardial infarction. In each chart the upper limit of the shaded area represents the concentration of total plasma cholesterol; the shaded area itself, the concentration of cholesterol as alpha lipoproteins; the part below the shaded area, the concentration of cholesterol as beta lipoproteins. The figures placed immediately below the shaded area are percentages of total cholesterol as alpha lipoprotein in Fraction IV+V+VI.

The percentage distribution of cholesterol in Fraction IV+V+VI rose from 3.2 to 27.6.

Later, an experiment for the purpose of observing the effects of smaller doses of methyl testosterone after the patient had been receiving 0.25 mg. estinyl daily showed that a dose of 10 mg. per day of the androgen exerted the same qualitative effects but was quantitatively somewhat less potent than the larger doses of 50 mg. The most evident change was a rise in the cholesterol-phospholipid ratio from 0.81 to 1.08. An increase in the dosage of estinyl to 1.0 mg. a day for five weeks had little effect in reversing the action of daily doses of 10 mg. of methyl testosterone.

Finally, a reduction of the methyl testosterone from 10 to 5 mg. per day with continuation of 0.5 mg. estinyl still indicated persistence of a neutralizing effect on the action of the estrogen. Although the percentage of cholesterol in Fraction IV+V+VI was increased from 8.0 to 14.5, the cholesterol-phospholipid ratios in unfractionated plasma and in Fraction I+III were again elevated to the levels obtained during the use of 10 mg. doses of methyl testosterone.

DISCUSSION

It appears that estinyl in relatively large doses (0.5–1.0 mg. o.d.) corrects several of the abnormalities of the plasma protein-lipid patterns in survivors of myocardial infarction. Invariably, it accomplishes a change in the distribution of lipoproteins, with increase in the concentration of alpha lipoproteins of Fraction IV+V+VI and a corresponding decrease in beta lipoproteins of Fraction I+III. Usually, there is a decrease in the plasma cholesterol and phospholipid concentrations and in the cholesterol-phospholipid ratio. It also causes a considerable change in the ratio of the concentration of cholesterol and phospholipids in Fraction I+III to the extent that the values are often less than 1.00. This suggests either unusual mixtures of lipoproteins or formation of protein-lipid combinations, presumably beta lipoproteins of a composition that varies from those recovered from Fraction I+III in normal subjects.

The action of oral estrone sulfate (premarin) in survivors of myocardial infarction appears to differ little from that of equivalent amounts of oral estinyl. An exact quantitative comparison of the effects of the two hormones is not possible because the trials are not sufficiently systematic or extensive. Single intravenous doses of 15–20 mg. of estrone sulfate are apparently without effect on either the distribution or concentration of the alpha and beta lipoproteins.

Other estrogens such as estradiol benzoate and diethylstilbestrol are also capable of modifying protein-lipid relationships of plasma, and their effects are qualitatively similar to estinyl and premarin. Any estimation of the relative influence of various estrogens on the concentration and distribution of lipoproteins would necessitate more extensive and differently organized experiments. Since qualitatively the actions of all of the estrogens tried in this study are similar, it appears that they are related to estrogenic activity and are not attributable to the chemical radicles of specific compounds. Their action is also apparently not limited to survivors of myocardial infarction.

In primary hypercholesterolemic xanthomatosis which may be regarded as the most severe form of lipid disturbance predisposing to atherosclerosis, the administration of estinyl is followed by predictable changes similar to those in survivors of myocardial infarction. Frequently, there is a reduction in the cholesterol concentration of plasma; invariably, there are changes in the distribution and a tendency to lowering of cholesterol-phospholipid ratios in plasma and in the beta lipoproteins of Fraction I+III. In no instance, however, is there complete restoration of normal protein-lipid patterns. As in survivors of myocardial infarction, the chemical effects of estinyl may be maintained as long as the hormone administration is continued; they disappear abruptly when treatment is discontinued.

Chemical changes following the use of estinyl and premarin in the nephrotic syndrome are difficult to evaluate because of the extremely variable course of the condition. Our observations of their effects in two subjects offer little hope, however, that the lipid abnormalities of the syndrome can be significantly altered by the use of estrogens. Although, qualitatively, the changes are similar to those observed in survivors of myocardial infarction and in patients with primary hypercholesterolemic xanthomatosis, they are quantitatively too small to be significant. Unfavorable alterations in the protein composition of plasma following the use of estinyl in one of the subjects discouraged trial of the hormone in other cases.

Methyl testosterone modifies protein-lipid relationships in plasma in a manner opposite to

that observed following the administration of estrogens. The most constant effect is in the distribution of cholesterol and consists of a relative decrease in alpha lipoproteins in Fraction IV+V+VI and relative increase in beta lipoproteins of Fraction I+III. Other changes often noted are an increase in the cholesterol-phospholipid ratio of the unfractionated plasma, and in Fraction I+III, and, occasionally, a decrease in Fraction IV+V+VI. A drop in the plasma cholesterol concentration is unpredictable. Even small amounts can exaggerate the abnormal protein-lipid pattern of a survivor of myocardial infarction. Intramuscular doses of testosterone propionate appear to have the same if not even greater effect upon protein-lipid relationships.

The antagonistic effects of estrogens and androgens are best shown when the two hormones are administered simultaneously. It is apparent that, with the doses used in these experiments, the action of the androgen, methyl testosterone, is far more potent than that of the estrogen estinyl. Complete obliteration of the estrogenic effects may be obtained by relatively small doses of methyl testosterone. Experiments devised to show the relative potency of the hormones under a great variety of conditions would be necessary before physiologic implications could be formulated.

It is noteworthy that, in the chick, Stamler and his associates[27] were able to contrive a combined dose of estrogen and androgen which had no feminizing effect but could protect against coronary artery lesions in cholesterol-fed chicks.

Under the conditions of our experiments, the effects of methyl testosterone following or during the administration of estrogen were greater than when the testosterone was given alone. Our data are, however, insufficient to establish a potentiating effect of the estrogen. Under the conditions of combined administration the deviations from normal accomplished by the androgen are greater than those seen in any pathologic conditions except hypercholesterolemic xanthomatosis or nephrosis and must be regarded as disturbing if not alarming.

SUMMARY

The concentration and the distribution of lipids bound as lipoproteins in plasma are modified by gonadal hormones.

The ingestion of estrogen in the form of ethinyl estradiol, estrone sulfate or diethylstilbestrol tends to correct the pathologic protein-lipid relationships of survivors of myocardial infarction, and in many instances accomplishes complete restoration of normal patterns.

In primary hypercholesterolemic xanthomatosis the effect of estinyl is qualitatively similar but is not sufficient to restore normal patterns.

In two cases of nephrosis chemical changes qualitatively similar to those observed in survivors of myocardial infarction were perceptible but were so small as to be therapeutically insignificant.

Methyl testosterone exerts the opposite chemical effect. It exaggerates the chemical pathology of survivors of myocardial infarction and can produce abnormalities in plasma protein-lipid relationships of individuals whose protein-lipid patterns have been previously normal.

When the estrogenic and androgenic hormones are given simultaneously, estrogenic effects are modified or obliterated. It has not been definitely demonstrated that the actions of testosterone are modified by estrogen.

The experiments demonstrate that lipoproteins are subject to chemical manipulation but do not explain the mechanisms by which gonadal hormones operate.

These observations appear to have possible significance in the pathogenesis of atherosclerosis. They indicate a chemical reason for the relative immunity of young women to the complications of atherosclerosis. They suggest that the action of estrogenic substances in atherosclerotic persons exhibiting striking protein-lipid abnormalities might be beneficial. They suggest that the use of methyl testosterone in such patients might be detrimental.

While the observations have certain therapeutic implications, they indicate side actions which will make the use of estrogens unacceptable to many patients. It is clearly established that the administration of these hormones eliminates chemical abnormalities which may be regarded as detrimental; however, more extensive clinical studies will be necessary before estrogens can be considered useful in the prevention of atherosclerosis or in the treatment of its complications.

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The Serum Lipoprotein and Cholesterol Concentrations of Central and North Americans with Different Dietary Habits*

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THERE is now strong evidence to indicate that both the quantity of food habitually eaten and its quality are related to the etiology of atherosclerosis in man. Although these cannot be the only determinants in the development of the disease process, both the caloric balance as determined by body weight[1–3] and the proportion of food calories derived from fat[4–6] have been found to correlate to a significant extent with the prevalence of clinical manifestations of atherosclerosis. It would be useful to apportion the relative importance of these dietary factors among other contributing causes since these are elements of the environment which can be altered by appropriate nutritional measures.

The chronic nature of atherosclerosis, coupled with the rigidity of human dietary habits, preclude a directed experiment but human cultural groups are sufficiently diverse to supply natural material for the investigation of these problems. Such a study has been undertaken with groups of North and Central American subjects which habitually consume very different diets. The serum lipid measurements of a rural Central American group have been compared with those of both urban Guatemalans and North Americans who consume much more protein, fat and cholesterol. Striking differences in the cholesterol levels and in the serum lipid patterns have been encountered.

PROCEDURES

Selection of Subjects. The Central American subjects were of two categories. Group A included persons of predominately Mayan Indian origin and cultural characteristics residing in rural, highland villages. Careful cultural, clinical and dietary observations had previously been carried out by workers from INCAP in the majority of the villages used in this study.[7–11] The individuals were all volunteers who were given a brief physical examination which included the measurements of height, weight, blood pressure and pulse rate. Those with acute or chronic diseases, and women who were pregnant or lactating were excluded when these conditions were revealed by the examination.

Group B included persons residing in either Guatemala City or Antigua and living in a favorable economic situation. The majority of the males were physicians, army officers or other business and professional men. The women were members of the families of children attending the "Guatemalan American School" in Guatemala City, the most expensive of the private schools. All had resided at least five years in Guatemala City and the majority were native born Guatemalans. These persons were also volunteers who appeared to be in good health. Measurements of height, weight, blood pressure and pulse rate were made and pregnant and lactating women were excluded.

The North American subjects taken for comparison are referred to as Group C. These were United States business and professional men and women, and office employees who were studied at the time of an annual health protection examination given by their organizations. The group does not include low income or poorly

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nourished individuals. These subjects were examined by a physician, a medical history was taken and the blood pressure recorded. A urine sample was examined for sugar and protein, and an electrocardiogram was taken. Subjects with evidences of acute or chronic disease were eliminated from the study. Those with a blood pressure exceeding 140/90, with an abnormal or questionable electrocardiogram, with an abnormal finding in the urine examination or with a history of cardiovascular disease were also excluded from the present considerations.

Dietary Methods. The diets of the Central American subjects of Group A were evaluated by seven-day family diet surveys collected by nutritionists familiar with the language, customs and foods of those people. These surveys were carried out in three of the six communities sampled, and in two other villages in the study area. In these surveys each family was visited daily and food portions were weighed when possible. The individual nutrient intakes were estimated using a food composition table based on the analysis of Central American foods[12] and with dietary allowances modified from those of the National Research Council (U. S.) to conform with local environmental conditions and mean body weights.[13] The data shown represent the findings in a combined sample of eighty-two families which, while not always included in the serum study, are believed representative of those subjects.

Randomly selected subjects in Group B, the urban Guatemalan business and professional persons, were requested to keep a record of their individual food consumption for a period of seven days. This was supplemented with personal interrogation when the questionnaires were returned. Fifteen of the persons cooperating were women, and eight were men.

The dietary approximations of the United States subjects of Group C were representative of those described in the literature.[14,15]

Laboratory Methods. The blood samples were collected in the field in Central America. These were immediately transported to the INCAP laboratory and the serum recovered and placed in labelled tubes for transport by air to Boston. Serum samples were kept at 0–5°C. during transport. The analyses were begun three to five days after the blood was drawn. Lipoproteins were measured with the ultracentrifuge by the method of Gofman et al.[16] Cholesterol was measured by the method of Abell et al.[17] The reliability of these methods in our hands has been described.[18]

Statistical Methods. The laboratory data for each group were first considered according to sex and decade of age. Comparison of means of these groups were made after a logarithmic transformation of the measurements in order to minimize skewness of the distributions.[19] This process leads to an asymmetric distribution of the arithmetic variance about the means so that both positive and negative standard deviations are shown.

The relative weights $\left(100 \times \frac{\text{observed weight}}{\text{ideal weight}}\right)$ were computed for all subjects on which height-weight data were available. For lack of more suitable reference data, the tables of Ideal Weight of the Metropolitan Life Insurance Company[20,21] were used for both the North and Central American groups. If the "ideal weights" of the Central Americans are in fact less than those of North Americans, this procedure would tend to overestimate the leanness of those subjects and to minimize rather than accentuate the differences upon which the conclusions have been based. The analyses of covariance[22] used to make adjustments for the differences of relative weight among the groups were applied to age ranges of subjects which were shown to have rectilinear slopes of the age-serum lipid regressions.

RESULTS

The diets of the subjects of Group A were very different from those of Groups B and C. The greatest single difference appeared to be the much lower proportion of fat calories in the largely vegetarian Central American group. A summary of the dietary habits of families in the five Central American villages measured is shown in Table I. These data are to be compared with the urban Guatemalan and North American dietaries summarized in the same table. The fat content of the diets of Group A subjects was comprised almost entirely of the intrinsic fat of the corn and other vegetable foods in the diet. There was little or no free or added fat. It will be noted from Table I that the diets of Groups B and C are similar.

Summaries of the serum lipid findings for the three groups are shown in Table II for males and females, respectively. The data are grouped according to age and the transformed means and the positive and negative variances computed as

anti-logs of the transformed measurements are shown. The evaluations of the significance of observed differences of means were done with the transformed data.

A striking characteristic of the data in Table II is the low serum cholesterol levels of the rural Guatemalans of Group A. This difference of cholesterol levels is the more remarkable because of the similarity of the lipoprotein levels of the three groups. When differences of the mean lipoprotein levels occurred among the groups, they were usually *increases* of the Central American levels above the North American level. This was particularly marked among the Group A females who showed elevation of all classes of lipoproteins above the North American level. The serum cholesterol levels of the business and professional groups of Guatemala and the United States were similar. In eight of the sixteen comparisons made, the lipoprotein levels of the urban Guatemalans were higher than those of age- and sex-matched North Americans. It was also of interest that there was no real difference of cholesterol levels between males and females among the rural Guatemalans of Group A.

The trends of lipoprotein levels with age were generally similar in all the groups. These showed a parabolic change with increasing age. Figure 1 illustrates these trends for the males and females, respectively.

The data were then examined for explanations of these observed differences. Since serum lipid levels have been shown to be positively correlated with relative weights in North American subjects by Dawber et al.[23] (see Walker[24]) and by Gofman and Jones,[25] it was of interest to study the relation of relative body weights to the serum lipid differences observed here. The relative weights of the Central American subjects of Group A were consistently less than those of the North Americans. (Table III.) These Central

TABLE I

DESCRIPTION OF THE STUDY GROUPS AND THEIR DIETARIES

Group	Number of Sera Analyzed		Occupation	Daily Intakes				
	Male	Female		Calories	Protein % of N.R.C. Allowance	Fat		Proportion of Calories from Fat (%)
						Animal (gm./day)	Vegetable (gm./day)	
A, rural Central Americans	112	91	Hand crafts, agriculture, household	2506*	125	13	8	8
B, urban Central Americans	92	71	Business, professional, household	2810†	141	68	43	36
C, urban United States	1,110	265	Business, professional, office workers	3250‡	134	140		40

* This intake represents 110% of requirements calculated on the basis of N.R.C. and FAO calorie recommendations for a "reference" level of activity.

† This represents 118% of the calculated calorie requirements and 117% of the protein recommended for males and 120% of the calories and 122% of the protein for females. In general these persons are much less active physically than those in Group A.

‡ It is remarkable that there are so few adequate dietary appraisals of well-nourished United States citizens. Extensive references to this kind of information may generally be traced to Department of Agriculture data on "food available for consumption." This is obviously not adequate for the present purposes but would seem to represent a correct order of magnitude.

Americans showed a downward trend of relative weight with increasing age in contrast to urban Guatemalans and North Americans whose trend of relative weight was upward with increasing age.

The covariance analysis which takes account of the third variable, relative weight, in the comparison of the two primary variables, environment and lipid levels, indicated that relative weight was of minor importance in the

TABLE II

SERUM LIPOPROTEIN AND CHOLESTEROL LEVELS OF CENTRAL AND NORTH AMERICAN SUBJECTS ACCORDING TO AGE AND SEX

(Concentrations are milligrams per 100 ml. serum; data not adjusted for body weight differences)

Age (yr.)	Group	A Rural Guatemala			B Urban Guatemala			C United States			F Ratio Significant ($p < 0.05$)*	Group Mean (differing from mean of Group C)†	
		N	$\bar{x}$	S.D.	N	$\bar{x}$	S.D.	N	$\bar{x}$	S.D.			
						I. Males							
20–29	S_f12–20	13	23	+11 −7	21	26	+24 −12	51	26	+ 21 − 12	−		
	S_f35–100		38	+37 −19		71	+66 −34		40	+ 40 − 20	+	B↑	
	Cholesterol		139	+24 −21		172	+65 −47		206	+308 −124	+	A↓	
30–39	S_f12–20	34	29	+17 −11	38	35	+24 −14	230	24	+34 −16	−		
	S_f35–100		41	+43 −21		87	+102 −47		54	+60 −28	+	A↓	B↑
	Cholesterol		143	+33 −27		210	+50 −41		218	+329 −131	+	A↓	
40–49	S_f12–20	18	27	+24 −13	22	34	+22 −14	490	36	+34 −18	−		
	S_f35–100		49	+69 −29		79	+62 −35		53	+57 −28	+	B↑	
	Cholesterol		144	+49 −37		186	+51 −40		233	+357 −141	+	A↓	
50–59	S_f12–20	16	26	+23 −12	8	42	+16 −12	254	34	+32 −16	−	A↓	B↑
	S_f35–100		35	+52 −21		81	+69 −37		47	+50 −24	+	A↓	B↑
	Cholesterol		136	+41 −31		210	+37 −32		232	+356 −140	+	A↓	
						II. Females							
20–29	S_f12–20	22	30	+20 −12	20	22	+19 −11	43	21	+16 −10	+	A↑	
	S_f35–100		47	+74 −28		32	+23 −14		16	+17 −8	+	A↑	
	Cholesterol		147	+34 −28		172	+52 −39		189	+69 −51	+	A↓	
30–39	S_f12–20	15	37	+24 −14	24	29	+18 −11	51	24	+15 −10	+	A↑	
	S_f35–100		58	+57 −29		41	+48 −22		18	+32 −11	+	A↑	B↑
	Cholesterol		156	+33 −28		200	+45 −36		203	+47 −39	+	A↓	
40–49	S_f12–20	11	39	+31 −18	16	28	+17 −11	108	27	+21 −11	+	A↑	
	S_f35–100		49	+75 −29		29	+26 −13		22	+28 −12	−	A↑	
	Cholesterol		172	+35 −29		208	+47 −38		219	+48 −29	+	A↓	
50–59	S_f12–20	9	39	+26 −16	10	59	+23 −17	48	33	+20 −13	+		B↑
	S_f35–100		41	+29 −17		73	+57 −32		31	+38 −27	+	A↑	B↑
	Cholesterol		138	+67 −46		222	+41 −35		247	+61 −48	+	A↓	

* Results of a test by the F ratio of variances of the probability that the observed differences of means of the three groups are greater than would be expected from sampling theory alone.

† Analysis of the group differences by the "t" test in order to identify which means differ with a probability of 5% or less that the observed difference is sampling error. The arrow indicates the direction of the difference from the mean of Group C.

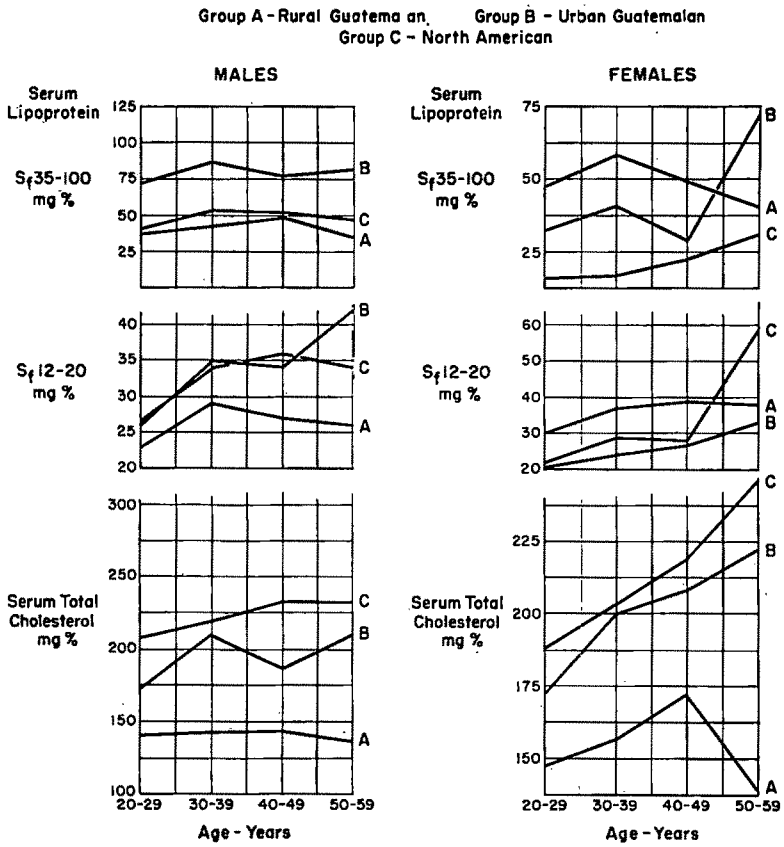


FIG. 1. A comparison of the levels of serum cholesterol and two classes of lipoproteins among subjects consuming different diets.

comparison. While the subjects of Group A, both male and female, were lighter than the age-matched subjects of either Group B or C, this fact did not greatly influence either the direction or magnitude of the observed differences of serum lipid components between the groups nor did this adjustment affect the conclusions drawn from these data.

COMMENTS

These observations indicate the presence of a distinct difference between the serum cholesterol levels of rural Guatemalan subjects and those of either urban Guatemalans or North Americans. The cholesterol levels of the rural Guatemalans are sufficiently different at all adult ages so that they would permit an accurate discrimination between the subjects using this characteristic alone. The serum beta-lipoprotein levels were in contrast to this difference for they were generally similar among all the groups and such differences as occurred were in either direction. The greater leanness of the Central Americans ap-

TABLE III

RELATIVE WEIGHTS $\left(\frac{\text{OBSERVED WEIGHT}}{\text{IDEAL WEIGHT}} \times 100\right)$ BY AGE AND SEX FOR RURAL GUATEMALANS, URBAN GUATEMALANS AND UNITED STATES SUBJECTS

Age (yr.)	Group A Rural Guatemalans $\bar{x}$	Group A Rural Guatemalans $SE_{\bar{x}}$	Group B Urban Guatemalans $\bar{x}$	Group B Urban Guatemalans $SE_{\bar{x}}$	Group C United States $\bar{x}$	Group C United States $SE_{\bar{x}}$	Group Mean (differing from mean of Group C)*
Males:							
20–29	96	± 2.77	98	± 2.84	107	± 1.82	A↓ B↓
30–39	96	± 1.54	107	± 2.19	107	± .73	A↓
40–49	93	± 1.89	109	± 2.35	110	± .45	A↓
50–59	76	± 2.00	106	± 4.95	111	± .88	A↓ B
Females:							
20–29	100	± 3.41	92	± 2.29	103	± 2.47	B↓
30–39	93	± 2.58	100	± 2.25	107	± 2.55	A↓ B↓
40–49	94	± 3192	105	± 3.75	106	± 1.55	A↓
50–59	83	± 3.33	105	± 5.38	106	± 2.53	A↓

*A↓—mean of Group A significantly < Group C ($p < 0.05$)
B↓—mean of Group B significantly < Group C ($p < 0.05$).

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pears to be of minor importance in determining the low serum cholesterol levels observed in that group. On the other hand if, as is suggested in Table III by the increasing relative weights at successive ages, the urban Guatemalan and North American subjects were not in caloric equilibrium but were slowly gaining weight, a part of the observed serum cholesterol difference might be attributed to this process of "becoming fat" rather than to simply being fat. This effect of positive caloric balance has been demonstrated in short-term experiments.[3] The present cross-sectional study will not provide further information on this point.

The interpretation of the observed lipoprotein differences is more complicated because the differences were both smaller in amount and variable in direction. The S_f 12–20 lipoprotein differences were in opposite directions for males and females, the male Central Americans of Group A often showed lower levels than the North Americans but the female Central Americans showed generally higher levels. Since the serum cholesterol and lipoprotein measurements are in part representative of the same materials and since a small but real correlation between the measurements has been demonstrated,[26,27] the direction of the differences found in the male groups was expected. The difference of cholesterol levels appears disproportionately greater, however, when appraised by the standards of experience with North American subjects.[28] The measurements of female Central American subjects of Group A are unique, for they combine low cholesterol levels with high lipoprotein levels when compared with age-matched North American females.

This unusual combination is rarely seen among North American subjects. We have collected a small series of similar measurements which we call the "Blumenthal pattern" after the first subject in whom it was seen.[29] In almost 10,000 observations of human serum we have found only seventy similar patterns. The ultracentrifugal appearance of such a serum is characterized by a low main peak (S_f 0–8) with an abundance of material in the region S_f 12–100. The sera are clear and the cholesterol level is generally below 200 mg. per cent. There are no obvious common patterns of diet or clinical description among the seventy subjects with this type of sera and the Central American subjects.

Inspection of the dietary evaluations of the rural Central American subjects indicates an average caloric intake which exceeds the National Research Council allowance for persons of this weight and environment by about 10 per cent. This observation taken with the relative weight data indicating that the Central Americans of Group A were on the average 15 per cent leaner (and we have probably underestimated this leanness by the use of North American height-weight standards) than either urban Guatemalans or North Americans implies that the differences must be accounted for by larger caloric expenditures in the rural Central Americans. Consideration of the activity requirements of people in such an agrarian culture indicates this is a reasonable explanation.

After extensive studies of such people, INCAP recomendations[30] for persons in Group A include 200 more calories daily for a 55 kg. man of age 25 and 500 more calories for a 50 kg. woman of this age than are recommended by the Calorie Committee of the Food and Agriculture Organization[31] and the National Research Council (U. S.).[32] It is then possible that the magnitude of caloric turnover is an important factor in determining the lipoprotein content of blood serum. This suggestion is also logical if we accept the hypothesis which places the serum lipoproteins as a spectrum of specialized lipid-bearing proteins along which dietary lipids are passed in preparation for tissue utilization.[33]

The data of Walker et al.[3] indicated that these lipoproteins and the serum cholesterol were significantly increased by a period of positive caloric balance even when this was obtained with a very low fat dietary intake. We may suppose that a large caloric intake, even low in fat, when balanced by a large caloric expenditure might selectively increase the serum lipoproteins since these must serve the physiologic transport function. The serum cholesterol level on the other hand is determined primarily not by the type or magnitude of the intake but rather by the nature of the caloric disposal. When the excess calories are stored as fat, the cholesterol level rises; and when the calories are distributed as heat and energy, the cholesterol level is low. This or some other mechanism of semi-independent control of cholesterol and lipoprotein levels in the blood seems necessary to explain the irregular correlation of these quantities in human blood which are observed in cross-sectional studies[18] among North American subjects.

On the basis of population studies, Keys has arrived at the conclusion that serum cholesterol levels are related to the presumed dietary intake of fat.[34] The present groups were selected for study with the intent of comparing persons consuming diets of widely varying fat content. The dietary appraisals confirmed the large magnitude of the differences in fat intake. The basic question is, therefore, whether the serum lipid differences observed can be explained, wholly or partially, by this dietary difference. Although the cholesterol differences encountered are consistent with a hypothesis relating cholesterol levels to fat intake, the differences of levels observed among the groups are not so readily explained because of the marked dissociation of serum cholesterol and lipoproteins observed among rural Guatemalans. Experimental manipulation of dietary lipids in human[3,33] and laboratory animal subjects[35] has not indicated such an effect. Most other human studies have not measured both the serum cholesterol and lipoprotein levels.

It still remains to be determined whether the Central American or similar material can resolve the uncertainty of the relative importance of the serum cholesterol and the serum lipoproteins in the pathogenesis of atherosclerosis. Since the Guatemalan subjects of Groups A and B appear to offer unique advantages in studying that question, anatomic material is now being gathered.

SUMMARY

1. The total cholesterol and beta-lipoprotein measurements of the blood sera of a group of rural Central American subjects subsisting on a largely vegetarian and very low fat diet have been compared with those of urban Guatemalan and North American subjects who habitually eat large amounts of fat.

2. The rural Central American subjects, both male and female, were shown to have lower mean cholesterol levels at all ages studied than did the other groups. There was little evidence of the typical North American increase of serum cholesterol level with age among these Central Americans. There was no sex-determined difference of mean cholesterol levels by decade among the rural Central American subjects. The cholesterol levels of urban Guatemalans living in a good economic status were similar to those of North Americans.

3. The serum lipoprotein differences observed among the groups were variable. They were only slightly and irregularly lower in the rural Central American males than in the North Americans and the beta-lipoproteins of the females of this group were frequently at *higher* levels than those of the North Americans. The urban Guatemalans showed lipoprotein levels as high or higher than those of the North American group.

4. These cholesterol and lipoprotein level differences could not be explained by the greater leanness of the Central Americans although this factor could be shown to have a small effect.

5. The greater leanness of the rural Guatemalan group taken with higher caloric consumption of that group suggests that the serum lipoprotein levels may be dependent upon the magnitude of energy turnover whereas the serum cholesterol levels are increased by energy accretion or fat deposition.

6. The differences in dietary fat intake among the groups do not serve to explain the serum lipid differences because they do not permit an explanation of the dissociation of cholesterol and lipoprotein measurements which is unique in the rural Guatemalans.

7. These Guatemalan populations appear to be a useful place to evaluate the relative importance of serum beta-lipoprotein and cholesterol levels in atherogenesis.

Acknowledgments: The authors wish to acknowledge the technical assistance of Miss Rita O'Connell, Miss Alice Wysocki, Mrs. Mary Jane Bullard and Miss Dorothy Bruno. Dr. Fabio Castillo planned and carried out the collection of the first groups of blood samples and the dietary surveys were done principally by Marina Flores, Emma Reh and Elisa Cruz.

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Analysis of 177 Cases of Hypertensive Vascular Disease with Papilledema*

One Hundred Twenty-six Patients Treated with Rice Diet

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THE prognosis in patients with hypertensive vascular disease and papilledema has always been grave. Until 1944[1,2] there was no medical treatment for this condition; since then a number of cases of malignant hypertension treated successfully with the rice diet have been reported.[3–15]

The purpose of this paper is to describe the clinical course of this disease as seen in 177 patients (treated and untreated) examined between October, 1942, and October, 1953, on our service. This series includes every patient with hypertensive vascular disease admitted during this period in whom the presence of neuroretinopathy was confirmed by fundus photographs. (Patients with chronic nephritis, i.e., history of albuminuria preceding hypertension, and polycystic kidney disease are not included.)

An attempt was made to treat all these patients with the rice diet. However, of these patients eighteen never actually took the rice diet. Sixteen of the eighteen are known to have died; in one no follow-up information is available. Only one of these eighteen patients, E. B., who was treated intermittently over a five and one-half months' period, is still living, nine years after she was first seen here. The clinical course in these patients was similar to that described by Wagener and Keith.[16] In their series of 146 patients with malignant hypertension without any special therapy only one survived five years and 79 per cent died within the first year after examination.

Thirty-three patients were willing to take the rice diet but this treatment had to be discontinued because they were unable to retain sodium or, in some cases, chloride in the serum when subjected to severe dietary restriction (less than 150 mg. sodium, less than 200 mg. chloride daily).

The rice diet was given for varying lengths of time to 126 patients. In three of these patients the follow-up information is incomplete and in three the follow-up period is too short, since treatment was started within the past year (these three are doing well). This leaves a total of 120 patients with malignant hypertension who were treated with the rice diet and in whom the effect of the rice diet on malignant hypertension can be studied. Fifty-two of these 120 patients are living.

Most of these patients, after an initial period usually of two to four months on the "basic" rice diet, received a "modified" rice diet which included small amounts of potato, cereals, non-leguminous and leguminous vegetables, meat, et cetera. In patients who continued under supervision the diet was increasingly liberalized as improvement permitted. Other patients discontinued the diet at various stages of treatment, but most of them continued to follow a salt-poor, fat-poor diet.

The urinary excretion of chloride, calculated as NaCl, on the basic rice diet, except in patients who are losing edema or who are acute or chronic salt losers) should be 5 to 15 mg. per 100 cc. urine (determined in a twenty-four-hour urine collection) if properly adhered to; on the "modified" rice diet, if correctly followed, it should be 15 to 25 mg. The chloride excretion in the urine therefore is a guide to whether or not the diet is being followed correctly.

Three of the 120 patients were between twenty and twenty-nine years of age, seventeen be-

* From the Department of Medicine, Duke University School of Medicine, Durham, N. C. This investigation was supported in part by a research grant from The National Heart Institute, National Institutes of Health, Public Health Service; and in part by the Anna H. Hanes Fund and by the Walter Kempner Foundation.

tween thirty and thirty-nine, fifty between forty and forty-nine, thirty-eight between fifty and fifty-nine, twelve between sixty and sixty-nine when the treatment was started. Of the fifty-two patients who are living two were between twenty and twenty-nine when the rice diet was started, five between thirty and thirty-nine, twenty-three between forty and forty-nine, sixteen between fifty and fifty-nine and six between sixty and sixty-nine. In our series age was not a significant factor in prognosis.

TABLE I
TOTAL PSP EXCRETION IN TWO HOURS AND SURVIVAL*

Total PSP Excretion in 2 Hours (%)	Number of Patients	Number Dead	Survived	
			Over 6 Months	Over 1 Year
Not done	1	1	0	0
0–15	15	15	1	0
16–25	7	7	0	0
26–35	8	8	0	0
36–45	1	1	0	0
Above 46	1	1	0	0
	33	33	1	0

* 33 patients: NaCl or Cl added to diet.

RENAL AND ELECTROLYTE STATUS

The two most important factors influencing the effectiveness of the rice diet in the treatment of malignant hypertension are the renal status (especially since salt-losing often precludes the use of the diet in patients with advanced renal involvement) and the length of time the patient is willing to adhere rigidly to treatment.

In Tables I to IV the survival times of the 120 patients treated with the rice diet and of the thirty-three patients to whom supplementary NaCl or Cl was given are correlated with the renal function and ability to conserve electrolytes: (1) Total PSP excretion in two hours (Tables I and II); (2) blood non-protein nitrogen concentration (Tables III and IV); (3) inability to maintain adequate sodium and/or chloride levels in the serum on the rice diet. (Tables I and III.)

Tables I and III list the thirty-three patients in whom drastic salt restriction was not carried out because of inability to conserve sodium and/or chloride in the serum. Tables II and IV list the 120 patients who were treated with the rice diet.

In Tables I and II the patients in both groups are listed according to the PSP excretion at the beginning of treatment. There were five patients who were critically ill in whom the PSP was not determined; all died in less than one year. There were twenty-eight patients with a total PSP excretion, in two hours, between 0 and 15 per cent. Twenty-six of the twenty-eight died in less than one year and none survived twenty-five months. Fifteen were salt losers receiving salt and thirteen were not, but with a total PSP excretion below 15 per cent in two hours the prognosis is nearly as bad in patients who do not lose salt as in those who do.

There were forty-seven patients in whom the total PSP excretion was between 16 and 35 per

TABLE II
TOTAL PSP EXCRETION IN TWO HOURS AND SURVIVAL*

Total PSP Excretion in 2 Hours (%)	Number of Patients	Number Dead	Survived One Year		Patients Living		
			Number	(%)	Number	(%)	Years (average)
Not done	4	4	0	0	0	0	...
0–15	13	13	2	15	0	0	...
16–35	32	21	19	59	11	34	3
36–55	34	19	26	76	15	44	4½
Above 56	37	11	34	92	26	70	5
	120	68	81	67	52	43	4

* 120 patients treated with rice diet.

cent in two hours. Nineteen (40 per cent) of the forty-seven patients survived one year; eleven (23 per cent) are still living with an average survival time, so far, of three years. Fifteen of the patients in this group were salt losers and received salt; all of them died.

There were thirty-five patients with a total PSP excretion between 36 and 55 per cent in two hours. Only one patient was a salt loser and received salt. Twenty-six (74 per cent) of the thirty-five patients survived one year; fifteen (43 per cent) are still living with an average survival time, so far, of four and one-half years.

There were thirty-eight patients with a total PSP excretion in two hours of 56 per cent or more. Only one patient was a salt loser and received salt. Thirty-four (89 per cent) survived one year; twenty-six (68 per cent) are still living with an average survival time, so far, of five years.

In Tables III and IV the patients in both groups are listed according to the initial non-protein nitrogen concentration in the blood. The non-protein nitrogen was over 60 mg. per 100 cc. of blood in thirty-seven patients. Twenty of the thirty-seven patients were salt losers and received salt; all twenty died. Of the seventeen who were treated with the rice diet, only three survived one year and only one (initial non-protein nitrogen 75 mg. per 100 cc. of blood; total PSP excretion in two hours 20 per cent) is still living four and one-half years after the start of treatment.

In eighty-seven patients the non-protein nitrogen was between 35 and 59 mg. per 100 cc. of blood. Twelve of these patients were salt losers and received salt; all twelve died. Seventy-five were treated with the rice diet. Fifty-five (63 per cent) of the eighty-seven patients survived one year; thirty-seven (43 per cent) are still living with an average survival time of four years.

TABLE III
INITIAL BLOOD NPN LEVEL AND SURVIVAL*

NPN (mg. per 100 cc. of blood) (Range)	Number of Patients	Number Dead	Survived	
			Over 6 Months	Over 1 Year
Over 100	6	6	0	0
75 to 99	6	6	0	0
60 to 74	8	8	0	0
50 to 59	3	3	0	0
35 to 49	9	9	1	0
Below 35	1	1	0	0
	33	33	1	0

* 33 patients: NaCl or Cl added to diet.

In twenty-nine patients the non-protein nitrogen was below 35 mg. per 100 cc. of blood. One patient was a salt loser and received salt. Twenty-three (79 per cent) of the twenty-nine patients survived one year; fourteen (48 per cent) are still living with an average survival time so far of four years.

The thirty-three patients who developed severe electrolyte imbalance are listed in Tables

TABLE IV
INITIAL BLOOD NPN LEVEL AND SURVIVAL*

NPN (mg. per 100 cc. of blood) (Range)	Number of Patients	Number Dead	Survived One Year		Patients Living		
			Number	(%)	Number	(%)	Years (average)
Over 100	4	4	0	0	0	0	...
75–100	5	4	1	20	1	20	4½
60–74	8	8	2	25	0	0	...
50–59	12	6	8	67	6	50	4½
35–49	63	32	47	76	31	49	4
Below 35	28	14	23	82	14	50	4
	120	68	81	67	52	43	4

* 120 patients treated with rice diet.

I and III; in these patients the rice diet treatment was discontinued. Development of marked electrolyte disturbance during drastic sodium and chloride restriction is a most ominous sign. Sodium chloride was given to fourteen during the first month of treatment, to seven during the second month and to seven during the third month. Five received chloride as NH_4Cl, KCl or HCl during the second to seventh week. All thirty-three patients died; twenty-seven died in Duke Hospital after an average of seventy-one days, and the other six died shortly after returning home (one to four months after first being seen in our clinic). In general, the likelihood of electrolyte loss on the rice diet is greater in patients with a low PSP excretion and/or marked azotemia. However, the inability to conserve electrolytes may, in some instances, be the chief manifestation of renal dysfunction and may occur in the absence of severe impairment of PSP excretion or of marked azotemia.

In contrast to surgical measures,[17–20] which are contraindicated when there is nitrogen retention or even moderate renal impairment (PSP excretion in the first fifteen minutes below 15 to 20 per cent), advanced renal impairment does not preclude an attempt at treatment with the rice diet and such treatment is not dangerous if the electrolyte balance can be controlled. The criteria for selection of patients for drug therapy have not yet been worked out; renal impairment, advanced age, cerebral and coronary artery involvement may under certain circumstances[21,22] contraindicate the use of hypotensive drugs; they do not contraindicate the use of the rice diet. However, Tables I to IV indicate that far advanced renal impairment seriously diminishes the chances of successful treatment with the rice diet, as with other forms of treatment of malignant hypertension.

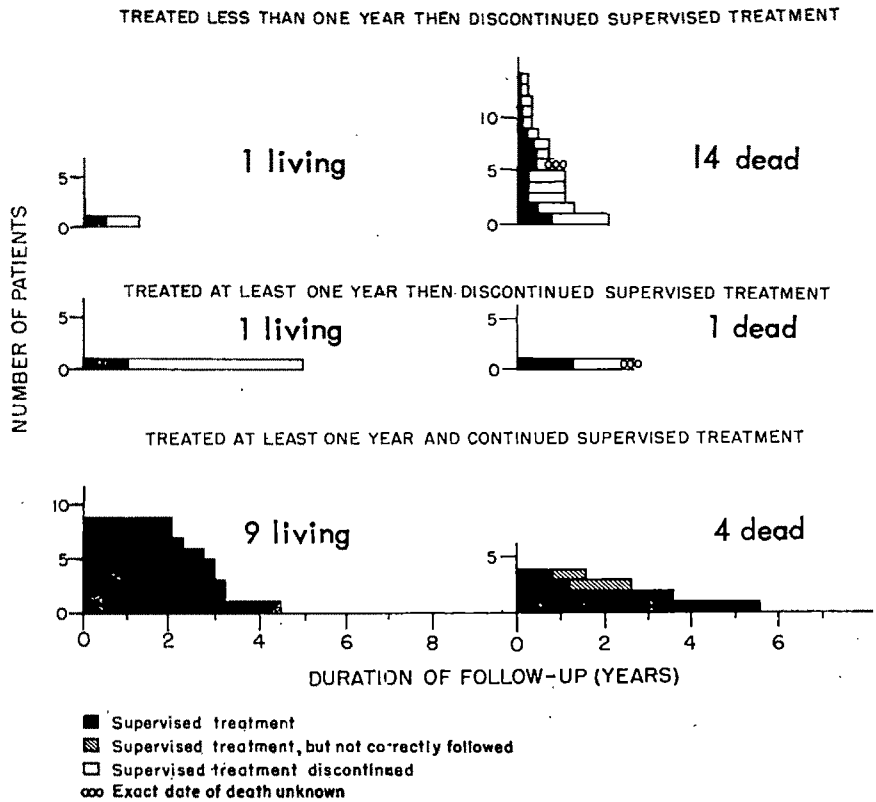


FIG. 1. Survival time and period of treatment (thirty patients, total PSP excretion in two hours, 35 per cent or less). Living, eleven patients; dead, nineteen patients.

TIME FACTOR

Since 1944[1] it has been known that the malignant phase of hypertensive vascular disease can be reversed by the rice diet; occasionally we have seen papilledema disappear within as short a time as one month, and six months of treatment with the rice diet is usually enough to cause

malignant hypertension either to revert to "benign" essential hypertension or to disappear completely.

However, the period of treatment required for modification of the underlying vascular disease and the degree to which it can be modified are more variable. In patients with the most severe involvement, uninterrupted maximal dietary treatment (as checked by a chloride excretion below 15 mg., expressed as NaCl, per 100 cc. of urine) may be necessary for years or in some cases perhaps indefinitely to maintain improvement. On the other hand, one patient, age twenty, had a normal blood pressure after two months on the rice diet, and her blood pressure is still normal five and one-half years after she had had malignant hypertension, although for the past three years she has been on a normal diet.

In Table v and Figures 1 and 2 the survival times are given according to the duration of treatment. With one exception (a patient who followed the diet poorly and died within eighteen months after first being seen in our clinic), the malignant phase of the disease had disappeared within one year in all patients treated for that length of time. The subsequent course of these patients was influenced by the severity of the underlying cardiovascular renal disease and the intensity and duration of treatment. In some patients improvement has been continuous; in others the vascular disease has remained stationary; in others it has progressed and cardiac or renal failure, coronary or cerebral artery disease or other complications have caused death. The data on the present status of the patients are given here but it is not within the scope of this paper to discuss the effect of rice diet therapy on the course of hypertensive vascular disease except when it is in the malignant phase.

There were one hundred patients (Table v)

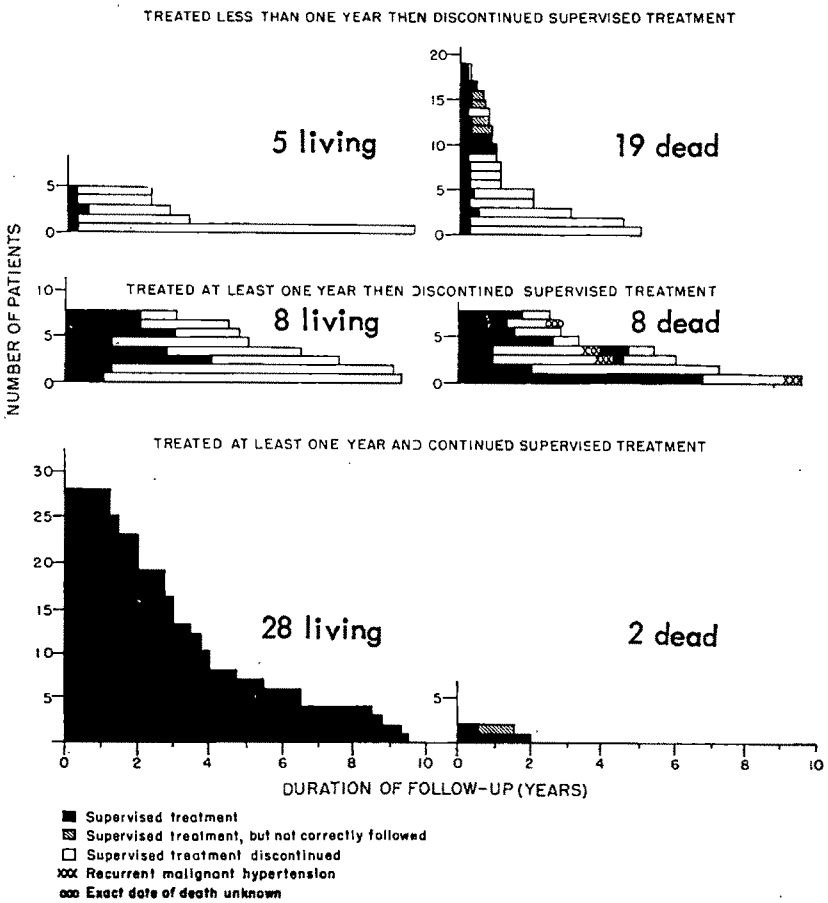


FIG. 2. Survival time and period of treatment (seventy patients, total PSP excretion in two hours, 36 per cent or more). Living, forty-one patients; dead, twenty-nine patients.

who were treated with the rice diet and were followed after the initial period of treatment; in these patients the relation of the length of time of supervised treatment to the survival time could be studied. (The eighteen patients who died in Duke Hospital during the initial period of treatment and two patients in whom the PSP was not determined are omitted.)

In thirty-nine of the one hundred patients, the period of supervised treatment was two to twelve months. Seven of the thirty-nine died during this period while supposedly still following the diet; only three of the seven were following it correctly. Thirty-two of the thirty-nine were treated for two to twelve months, and then to a greater or lesser extent discontinued the treatment; only six (19 per cent) of the thirty-two are still living.

Eighteen of the one hundred patients were treated one to seven and one-half, average three, years and then to a greater or lesser extent discontinued the treatment. Nine of these (50 per cent) are still living. In three instances malignant hypertension recurred two and one-half to three and one-half years after discontinuing treatment, and in two of the three papilledema again disappeared during a second period of treatment with the rice diet, which was again discontinued; in one the malignant phase could not be reversed a second time; all three died. The nine who are living had been treated with the rice diet, strict and modified, for an average of three years before discontinuing it; they have survived an average of four years since they discontinued the diet. Two are being treated elsewhere for heart failure, five have continued on a "salt-poor, fat-poor" diet; two have resumed a general diet.

Forty-three of the one hundred patients continued treatment with the rice diet; they have been treated one to nine and one-half, average four years. Thirty-seven (86 per cent) of these are still living; of the six who died, only three were following the diet correctly.

TABLE V

RELATION OF LENGTH OF TIME OF SUPERVISED TREATMENT WITH RICE DIET TO SURVIVAL*

Time of Supervised Treatment	Number of Patients	Survived One Year		Patients Living		
		Number	%	Number	%	Years (average)
Less than 1 year	39†	20	51	6	15	3
Over one year:						
Supervised treatment discontinued (after 1 to 7½, average 3 years)	18	18	100	9	50	4
Supervised treatment continued (1 to 9½, average 4 years)	43‡	43	100	37	83	4

* 100 patients treated with rice diet.

† Seven of these patients died while supposedly following the rice diet; only three of the seven were following the diet correctly.

‡ Of the six patients who died after the first year of treatment, only three were following the diet correctly.

Since, as has already been indicated, there is a marked difference in survival of patients with a total PSP excretion below 35 per cent in two hours as compared to those with a total PSP excretion of 36 per cent or more in two hours, the effect of the time factor was studied in these two groups of patients separately.

Thirty (Figure 1) of the one hundred patients had a PSP excretion (in two hours) below 35 per cent. Fifteen of these patients were treated six months or less. Eight died within the first year; six died after one to two, average one and one-half years; one is still living one year after treatment was started.

Two patients discontinued treatment after one and one-half and two and one-half years, respectively; the first one died shortly thereafter; the other is still living two and one-half years after discontinuing supervised treatment. Thirteen have continued treatment from one to five and one-half, average three years; nine (69 per cent) are still living. Of the four who died while supposedly still following the diet, two were following it well as shown by a chloride

excretion, expressed as NaCl, usually below 25 mg. per 100 cc. of urine (as compared to 5 to 15 mg. on the basic and 15 to 25 mg. on the correctly followed "modified" rice diet); two adhered to the diet poorly, chloride excretion 44 mg. per 100 cc. of urine, average of the last five determinations in each of the patients. The nine who are still alive do not at all times follow the diet correctly; the average chloride excretion in the urine has been 25 to 40, average 32 mg. per 100 cc. (average of the last five to fifteen determinations in each instance). These patients continue to return here for examination and, if indicated, for short periods of strict treatment several times a year.

Seventy patients (Figure 2) had a total PSP excretion of 36 per cent or more in two hours. In twenty-four the period of controlled treatment under our supervision was less than one year. Seven of the twenty-four died while supposedly still on the diet after six to nine, average seven and one-half months of dietary treatment; three were following the diet well (chloride excretion usually below 25 mg. per 100 cc. of urine); two were following the diet moderately well (chloride excretion 36 mg. per 100 cc. of urine, average of the last three determinations in each instance); one was not adhering well to the diet (chloride excretion 53 mg. per 100 cc. of urine, average of the last five determinations); one had been given small amounts of toast and other additions to increase the salt content of the diet during the fourth month of treatment when electrolyte imbalance developed. The other seventeen patients in this group were treated less than six months; thirteen survived one year and five are still living (three years average after first being seen at our clinic).

In forty-six patients the period of controlled treatment under our supervision was at least one year. Sixteen discontinued the diet after one to seven and one-half years: eight have died; eight are still living.

Thirty continued to follow the rice diet, strict and modified: twenty-eight (93 per cent) are still living, one to nine and one-half, average four, years after first being seen here. Two died while supposedly still on the diet: one was following the diet well (chloride excretion usually below 20 mg. per 100 cc. of urine), the other was following the diet only moderately well (chloride excretion 35 mg., average of ten determinations).

TABLE VI

RETINOPATHY IN 100 PATIENTS TREATED WITH RICE DIET*

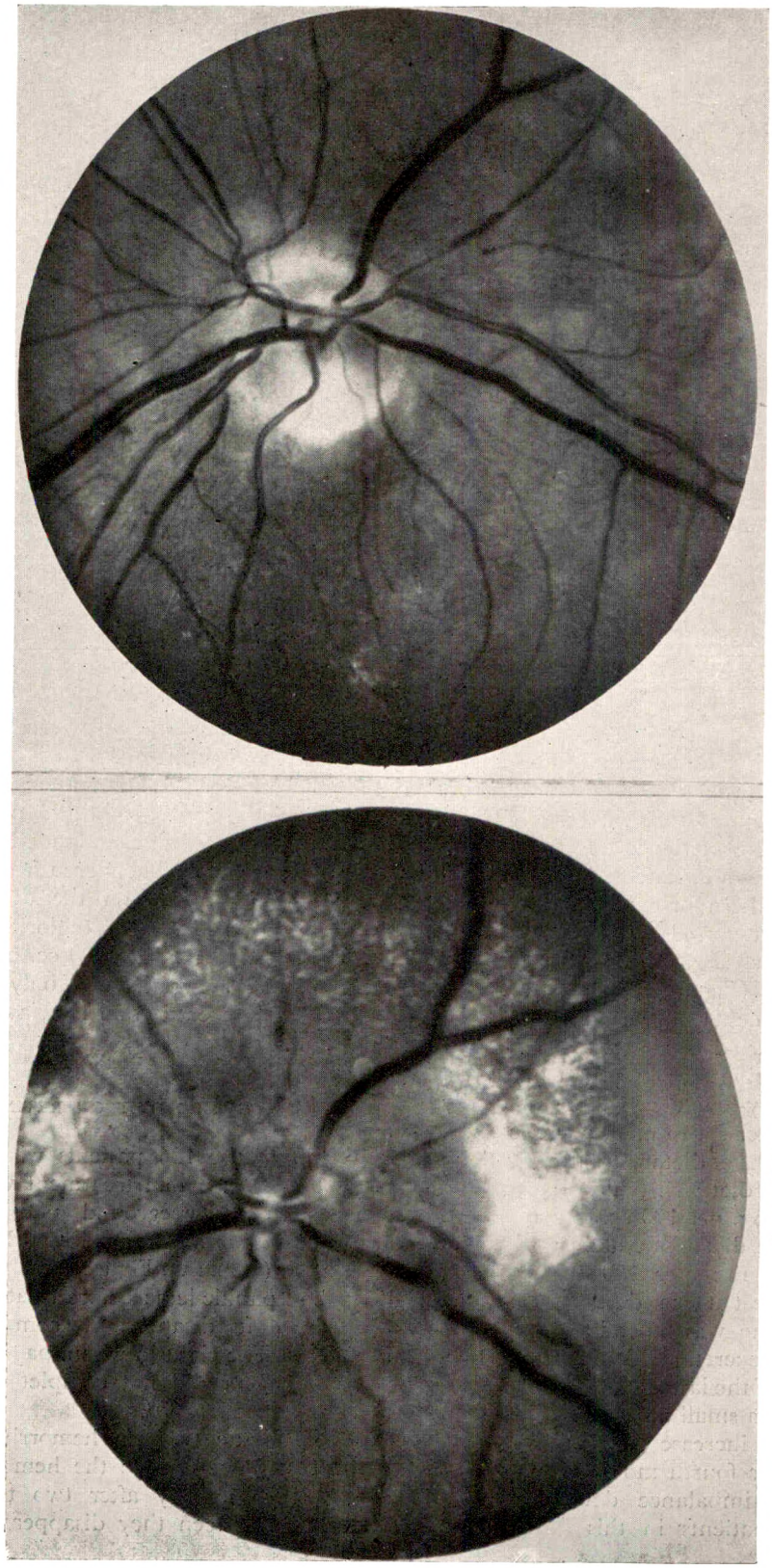
	Disappeared Completely		Disappeared Partially		No Change	
	Number of Patients	Time in Months (averages)	Number of Patients	Time in Months (averages)	Number of Patients	Time in Months (averages)
Papilledema (100 patients)	92	5	3	2	5	4
Hemorrhages (90 patients)	69	8	16	8	5	4
Exudates (94 patients)	56	15	30	7	8	5

* Eyeground photographs available before and 1 to 121 months after treatment with the rice diet was started.

EFFECT OF RICE DIET ON RETINOPATHY

In 100 of the 126 patients with malignant hypertension in whom treatment with the rice diet was used, eyeground photographs are available before and after 1 to 121 months of treatment. (Table VI, Figure 3.) All the 100 patients had papilledema before treatment and in ninety-two it disappeared completely with treatment. The eight in whom papilledema was not affected or did not completely disappear died.

Ninety patients had hemorrhages before treatment. In sixty-nine the hemorrhages disappeared completely after two to thirty-one months. In sixteen they disappeared partially



July, 1954

June, 1944

FIG. 3. Ten year follow-up in malignant hypertension (A. A. H., man, age 47 to 57).

after two to thirty-one months. In five there was no change after two to eight months. In two patients hemorrhages developed during treatment but were not present at the most recent examination. In three patients in whom hemorrhages had disappeared, they recurred and were present at the most recent examination.

2 to 6 mg. (two patients), apresoline® 50 to 150 mg. (twelve patients; one patient has taken as much as 400 mg. of apresoline at times), hexamethonium chloride 250 to 1500 mg. (eleven patients), and raudixin 50 to 150 mg. (nine patients).

Table VII shows the length of treatment with

TABLE VII

FIFTEEN PATIENTS GIVEN HYPOTENSIVE DRUGS

Initials	Fundi before Diet P H E†	Length of Dietary Treatment before Drugs Were Started (mo.)	Chloride Excretion before Drugs (as NaCl mg. per 100 cc. of urine)*	Fundi before Drugs Added P H E†	Medication at Most Recent Examination (mg. daily)			Fundi at Most Recent Examination P H E†
					C_6	Apresoline	Raudixin	
E. N.	+++	30	30	0 0 0		none‖		0 0 0
J. N.	+0+	30	40	0 0 0	1000	100		0 0 0
H. G.	+++	24	28	0 0 0			100	0 0 0
H. L.	+++	21	37	0 0 0		100		0 0 0
L. M.	+++	20	21	0 ++			100	0+0
J. M.	+++	18	105	0 ++		75	100‡	0 0 0
W. L.	+++	17	63	0 0 0		none‖		0 0 +
W. R.	+++	14	36	0 0 +		none‖		0 0 +
B. T.	+++	14	23	0 0 0			150	0 0 0
L. R.	+++	9	36	0 ++		200–400		0 0 0
P. C.	+++	9	26	0 ++	750	75		0++
J. C.	+++	8	40	0 ++	750	100	150	0 0 0
J. W.	+0+	6	34	0 ++	750		150	0 0 0
J. K.	+++	½		+++		none‖		0 0 +
M. B.§	+++	0		+++		100	150	0 0 0

* Should be 5 to 15 mg. per 100 cc. of urine on the basic rice diet and 15 to 25 mg. on the correctly followed "modified" diet.

† PHE stands for papilledema, hemorrhages, exudates.

‡ 3 mg. veraloid plus medication as listed.

§ Had received 25 mg. veraloid before she came to us, dose cut to 6 mg. during first four months, then all drugs stopped and resumed twenty-eight months later. (Chloride excretion 33 mg. per 100 cc. of urine before drugs restarted.)

‖ Had received 50–100 mg. apresoline, 500–1000 mg. hexamethonium, or 100 mg. raudixin singly or in combination for two to ten months, but were not receiving medication at most recent examination.

Ninety-four patients had exudates before treatment. In fifty-six the exudates disappeared completely after two to thirty-four months. In thirty they disappeared partially after one to twenty-two months; in eight there was no change after one to eleven months. In five patients in whom the exudates had disappeared, they were again present at the most recent examination.

After zero to thirty-six, average fifteen, months of treatment by diet alone, fifteen of the one hundred patients were given hypotensive drugs in addition to the strict or modified rice diet for three to thirty, average fourteen, months. The drugs were used singly or in combination. The doses per day were: veraloid

the rice diet before drugs were added, the funduscopic findings before treatment with the rice diet, before the drugs were given and at the most recent examination, and the present medication.

These drugs in the doses in which we have used them have caused a further lowering of the blood pressure in some instances but did not alter the course of the retinopathy nor was there any improvement of hypertensive heart or kidney disease noted which could be attributed to them. Treatment with the drugs was started in only two instances before the disappearance of papilledema and these fifteen patients, therefore, have been included with the other patients.

EFFECTS OF RICE DIET ON PATIENTS FOLLOWED FOR AT LEAST ONE YEAR

There are sixty-one patients who were re-examined here at least one year after treatment with the rice diet had been started. These sixty-one patients include fifty-seven of the sixty-one patients listed as treated for over one year in Table v, three patients who are listed as treated for less than one year but who were re-examined here one year after treatment with the rice diet had been started, and one patient who was followed here for thirteen months but whose present condition is not known. Of the sixty-one patients eighteen had a total PSP excretion of 16 to 35 per cent in two hours; twenty-three had a total PSP excretion of 36 to 55 per cent in two hours; 20 had a total PSP excretion of 56 to 88 per cent in two hours. The effects of the rice diet on blood pressure, serum cholesterol, blood non-protein nitrogen, T_1 in the electrocardiogram, cardiothoracic ratio and renal function are shown in Tables VIII to XIV. A comparison is made of the findings during the period of "strictest adherence to the rice diet" with those at the most recent examination.

TABLE VIII
BLOOD PRESSURE IN SIXTY-ONE PATIENTS TREATED WITH RICE DIET*

Before Treatment (average)	During Period of Strictest Adherence to Rice Diet (average †)	At Most Recent Examination (average ‡)
219/134	168/105	184/109

* Followed for at least one year.
† After 2 to 13, average 4, months.
‡ After 1 to 9½, average 4, years.

Even during the period of "strictest adherence to the rice diet" determinations of the chloride excretion in the urine showed that some patients were deviating from the prescribed diet; the chloride excretion of these sixty-one patients during the period of "strictest adherence to the rice diet" was 5 to 52, average 18, mg. per 100 cc, expressed as sodium chloride.

Table VIII lists the average blood pressures before treatment, during the period of "strictest adherence to the rice diet," and at the most recent examination. The average blood pressure of these sixty-one patients decreased from 219/134 to 168/105 during the period of "strictest adherence to the rice diet." In eighteen (30 per cent) of the sixty-one patients the blood pressure decreased to 149/99 or below; in eight (13 per cent) of the sixty-one to 125/89 or below during the period of "strictest adherence to the rice diet."

TABLE IX
BLOOD NPN IN FORTY PATIENTS TREATED WITH RICE DIET*

Range before Treatment (NPN mg. per 100 cc. of blood)	Number of Patients	Before Treatment (averages)	During Period of Strictest Adherence to Rice Diet (averages †)	At Most Recent Examination (averages ‡)
36–45	22	39	33	32
46–75	18	53	36	45

* Followed for at least one year. Initial NPN 36 mg. or more per 100 cc. of blood.
† After 2 to 9, average 4, months.
‡ After 1 to 9½, average 3, years.

Thirty-two of the sixty-one patients were following the rice diet well or moderately well at the most recent examination (chloride excretion, as NaCl, 24 mg. per 100 cc. of urine, average of the most recent determinations, one to eight and one-half, average five, years after the rice diet had been started). The average blood pressure of these thirty-two patients at the most recent examination was 175/105 as compared to 217/132 before treatment and 165/104 after the first two to six, average four, months on the rice diet.

The other twenty-nine patients followed the diet moderately well to poorly most of the time since the initial period of strict treatment here (chloride excretion, as NaCl, 65 mg. per 100 cc. of urine, average of most recent determinations, 1 to nine and one-half, average three, years after the rice diet was started). The average blood pressure of these twenty-nine patients at the most recent examination was 193/114 as compared to 220/136 before treatment and 170/106 after the first two to thirteen, average five, months on the rice diet.

In fifty-nine of the sixty-one patients blood NPN determinations are available before treatment, during the period of "strictest adherence to the rice diet," and at least one year after treatment was started. The NPN was elevated in forty of these patients and Table IX shows the changes during treatment. In thirty-three (82 per cent) it was lower at the most recent examination than it had been initially. Three patients, who had had a normal NPN before

the rice diet was started, developed azotemia. All three had followed the diet poorly, chloride excretion 92 mg. per 100 cc. of urine, average of four to seven determinations each; the NPN, average, was 32 mg. per 100 cc. of blood before treatment, 31 mg. after the first four months, average, on the rice diet and 51 mg. two to five, average three, years after treatment with the rice diet had been started.

TABLE X

SERUM CHOLESTEROL IN FORTY-SIX PATIENTS TREATED WITH RICE DIET*

Serum Cholesterol Level	Number of Patients	Serum Cholesterol (mg./100 cc.)		
		Before Treatment (averages)	During Period of Strictest Adherence to Rice Diet (averages†)	At Most Recent Examination (averages‡)
Increased.....	6	259	233	330
Decreased:.....	40	288	199	200
to 219 or below...	32	282	192	183
to 220 or above...	8	312	230	266

* Followed for at least one year. Initial cholesterol concentration in serum 220 mg. per 100 cc. or above.
† After 2 to 9, average 4, months.
‡ After 1 to 7, average 3, years.

In fifty-five of the sixty-one patients the cholesterol concentration in the serum was determined before treatment, during the period of "strictest adherence to the rice diet" and one to seven years after the treatment had been started.

Forty-six (84 per cent) of these fifty-five patients had a serum cholesterol concentration of 220 mg. per cent or more when first seen here, and the data on these patients are shown in Table x. Only two of these forty-six patients had an increase in the serum cholesterol concentration during treatment with the strict rice diet; the average cholesterol concentration in these two patients was 257 mg. per 100 cc. of serum before treatment and 290 mg. after the first four months, average, on the rice diet.

In six patients (13 per cent) the serum cholesterol was higher at the most recent examination three to six, average four, years after treatment with the rice diet was started than before. In forty (87 per cent) of the forty-six patients with an initial cholesterol concentration in the serum above 220 mg. the cholesterol concentration in the serum decreased; in thirty-two (70 per cent) of the forty-six it decreased to 219 mg. per 100 cc. or below. In two of the nine patients who had had a cholesterol concentration in the serum of 219 mg. or below per 100 cc. before treatment, the cholesterol concentration in the serum increased to 220 mg. or above. In these two patients the cholesterol concentration in the serum, average, was 215 mg. per 100 cc. before treatment, 212 mg. after the first three months, average, on the rice diet and 252 mg. two and one-half years, average, after treatment had been started.

TABLE XI

T_1 IN THE ELECTROCARDIOGRAM IN FORTY-NINE PATIENTS TREATED WITH RICE DIET*

	Before Treatment (Number of Patients)	During Period of Strictest Adherence to Rice Diet (Number of Patients)	At Most Recent Examination (Number of Patients)
T_1 upright....	14	38	28
T_1 diphasic....	10	4	5
T_1 inverted....	25	7	15†

* Followed for at least one year.
† One patient who was following the diet poorly developed a left bundle branch block.

In forty-nine of the sixty-one patients electrocardiograms are available for comparison (no digitalis, no infarction, etc.) before treatment, during the period of "strictest adherence to the rice diet" and one to nine and one-half years after treatment had been started. (Table XI.) In thirty-five (71 per cent) T_1 was abnormal before treatment; in ten it was diphasic; in twenty-five it was inverted.

In fourteen patients T_1 was upright before the rice diet was started; in none of the fourteen was there a change in the direction from upright to inverted after one and one-half to eight and one-half, average four, years. In nine of the ten patients in whom T_1 was diphasic before treatment it became normally upright after three to thirty-two, average eight, months of adequately followed dietary treatment. In one patient, it remained diphasic. In seven of the twenty-five patients in whom T_1 was inverted before treatment it remained inverted after one to three, average two, years. In eighteen (72 per cent) of the twenty-five patients there was a change in the direction from inverted to upright; in three T_1 became diphasic after seven to ten, average eight, months; in fifteen it

became normally upright after three to seventeen, average eight, months.

In twelve of the twenty-seven in whom the electrocardiogram had initially shown improvement it again became more abnormal. Nine of the twelve were following the diet poorly. In one of the nine patients a left bundle branch block developed.

In forty-nine of the sixty-one patients comparable chest films (no digitalis) are available before treatment, during the period of "strictest adherence to the rice diet" and at the most recent examination one to nine and one-half, average three, years after treatment had been started. (Table XII.) Before treatment the cardiothoracic ratio was 0.42 or below in only three patients (6 per cent); during the period of "strictest adherence to the rice diet" it was 0.42 or below in twenty-four (49 per cent). The cardiothoracic ratio before treatment was over 0.48 in twenty-nine (59 per cent); in nineteen it was 0.48 to 0.52 and in ten it was 0.53 to 0.59. During the period of "strictest adherence to the rice diet," the cardiothoracic ratio was over 0.48 in only seven (14 per cent). In these seven patients the average cardiothoracic ratio had decreased from 0.54 to 0.49 after the first five months, average, on the rice diet.

In thirty-six (73 per cent) of the forty-nine patients the decrease in cardiothoracic ratio during the period of "strictest adherence to the rice diet" was more than 10 per cent. In twenty-one of these thirty-six patients the cardiothoracic ratio at the most recent examination had again increased by 10 per cent or more. Fourteen of the twenty-one patients in whom cardiomegaly recurred were following the diet poorly; the cardiothoracic ratio, average, in these fourteen patients increased from 0.43 after the first four months, average, on the rice diet to 0.50 at the most recent examination. In the seven patients who were following the diet "satisfactorily" and in whom cardiomegaly recurred, the cardiothoracic ratio increased from average figures of 0.40 to 0.46.

TABLE XII

CARDIOTHORACIC RATIO IN FORTY-NINE PATIENTS TREATED WITH RICE DIET*

Initial Cardiothoracic Ratio (range)	Before Treatment		During Period of Strictest Adherence to Rice Diet		At Most Recent Examination	
	Number of Patients	CT Ratio (averages)	Number of Patients	CT Ratio (averages†)	Number of Patients	CT Ratio (averages‡)
0.36 to 0.42	3	0.41	24	0.40	12	0.41
0.43 to 0.47	17	0.45	18	0.45	16	0.46
0.48 to 0.52	19	0.50	6	0.49	16	0.50
0.53 to 0.59	10	0.56	1	0.53	5	0.55
0.36 to 0.59	49	0.49	49	0.43	49	0.46

* Followed for at least one year.
† After 1 to 22, average 6, months.
‡ After 1 to 9½, average 3, years.

TABLE XIII

KIDNEY FUNCTION (PSP EXCRETION) IN FORTY PATIENTS TREATED WITH RICE DIET*

	Number of Patients	Total PSP Excretion in 2 Hours (%)		Length of Time in Months (averages)
		Before Treatment (averages)	At Most Recent Examination (averages)	
Diet poorly followed (after initial period):	14	49	43	48
Increased or unchanged.....	5	52	59	45
Decreased.....	9	47	35	50
Diet well followed:	26	44	47	46
Increased or unchanged.....	17	43	54	51
Decreased.....	9	44	37	38

* Followed for at least two years.

Table XIII shows the change in total PSP excretion in two hours in forty patients treated with the rice diet, strict and modified, for two years or longer. In nine (64 per cent) of

the fourteen who followed the diet poorly after the initial period of treatment, the PSP excretion decreased; in five (36 per cent) it increased In nine (35 per cent) of the twenty-six who followed the diet well it decreased; in seventeen (65 per cent) it increased.

TABLE XIV
KIDNEY FUNCTION (PSP EXCRETION) IN TWELVE PATIENTS TREATED WITH RICE DIET*

	Number of Patients	Total PSP Excretion in 2 Hours (%)	
		Before Treatment (averages)	At Most Recent Examination (averages)
Diet poorly followed (after initial period)	5	53	44
Followed diet well	7	52	60

* Followed for at least five years.

There are twelve patients in whom repeated determinations of the total PSP excretion in two hours are available after five years or more of observation. (Table XIV.) Five followed the diet poorly; the average PSP excretion in two hours of these five patients decreased from 53 to 44 per cent. Seven have adhered to the diet well; in these seven the average PSP excretion in two hours has increased from 52 to 60 per cent.

It usually takes at least three to four months of intensive dietary treatment to produce a significant decrease in the heart size and blood pressure and it frequently takes over one year for the inverted T_1 in the electrocardiogram to become normally upright. For improvement in kidney function at least two to three years are needed.

COMMENT

When treatment with the rice diet was started in 1939 it was thought that extreme dietary restriction would have to be continued indefinitely to compensate for an underlying renal metabolic dysfunction. At present we have quite a few patients who have been able to resume a salt-poor, fat-poor diet and some who, after an adequate period of intensive treatment first with a strict and then with a modified rice diet, have been able to tolerate a general diet without recurrence of vascular disease.

In patients with malignant hypertension treated with the rice diet before the PSP excretion in two hours has decreased to less than 35 per cent, the prognosis is no longer as unfavorable as it was. Sixty (84 per cent) of the seventy-one patients with a total PSP excretion of 36 per cent or more in two hours survived one year (Table II) and had overcome the malignant phase of the hypertensive vascular disease. Of these sixty patients, thirty (Fig. 2) continued to follow the rice diet; twenty-eight (93 per cent) of the thirty are still living four years after initiation of treatment. However, since most cases of malignant hypertension are preceded by known "benign" hypertensive vascular disease, malignant hypertension can now be regarded as a preventable condition. Hypertensive vascular disease, no matter how mild the symptoms, should be treated immediately and vigorously as soon as the diagnosis has been established, and in those patients who have already developed the malignant phase and have been successfully treated intensive dietary treatment should not be discontinued before the underlying disease has disappeared.

SUMMARY

One hundred seventy-seven patients with hypertensive vascular disease with papilledema (confirmed by eyeground photographs) were seen between October, 1942, and October, 1953. In six patients follow-up information is inadequate or the treatment period too short for evaluation.

In thirty-three patients with hypertensive neuroretinopathy who were willing to be treated with the rice diet, electrolyte imbalance developed within the first three months on the rice diet. Treatment with drastic salt restriction therefore could not be carried out, but additional NaCl or other chloride was given. In thirty-one of these thirty-three patients the total PSP excretion in two hours was below 36 per cent. All thirty-three patients died.

In eighteen patients treatment with the rice diet was not carried out at any time; only one of these is still living.

In 120 patients with hypertensive neuroretinopathy treatment with the rice diet was carried out. The period of treatment was from 1 to 117 months. In 83 of the 120 patients the

total PSP excretion in two hours was below 55 per cent. (This includes four patients in the terminal stage of the disease, in whom the PSP was not measured.) Forty-seven (57 per cent) of these patients survived one year; twenty-six (31 per cent) are still living four and one-fourth years (average) after treatment was started. In thirty-seven patients the total PSP excretion was 56 per cent or more in two hours; thirty-four (92 per cent) survived one year; twenty-six (70 per cent) are still living five years (average) after treatment was started.

One hundred of the 120 patients were followed after the initial period of the treatment with the rice diet, and in these patients the relation of the length of time of supervised treatment to the survival time could be studied. In 39 of the 100 patients the period of supervised treatment was two to twelve months. Seven of the thirty-nine died during this period while presumably still following the diet; only three of the seven were following it correctly. Thirty-two of the thirty-nine were treated for two to twelve months, and then to a greater or lesser extent discontinued the treatment; only six (19 per cent) of the thirty-two are still living.

Eighteen of the 100 patients were treated one to seven and one-half, average three, years and then to a greater or lesser extent discontinued the treatment. Nine of these (50 per cent) are still living.

Forty-three of the 100 patients were treated over one year with the rice diet and continued treatment. Thirty-seven (86 per cent) of these are still living; of the six who died, only three were following the diet correctly.

The effect of the rice diet on retinopathy was studied in 100 patients in whom eyeground photographs were available. In ninety-two of the 100 patients, papilledema disappeared completely.

The effects of the rice diet on blood pressure, serum cholesterol, blood non-protein nitrogen, T_1 in the electrocardiogram, cardiothoracic ratio and renal function were studied in sixty-one patients who were re-examined here at least one year after treatment had been started. A comparison of the findings before treatment, during the period of "strictest adherence to the rice diet" and at the most recent examination shows that the "strictest adherence to the rice diet" produces the most favorable results. Dietary modifications therefore should be made gradually and with careful observations of blood pressure readings, blood chemical findings, electrocardiograms, chest films, eyeground photographs, etc.

The results are interpreted as indicating that intensive dietary treatment should be started as early as possible and continued until all signs of the vascular disease have disappeared.

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Effects of Restriction of Dietary Fat and Cholesterol upon Serum Lipids and Lipoproteins in Patients with Hypertension*

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THE effects of drastic restriction of dietary fat intake and elimination of cholesterol intake upon serum lipid patterns in patients with hypertension have been presented previously as part of an investigation of the Kempner rice diet.[1] This therapeutic diet, which contains no cholesterol, practically no fat and only minimal amounts of protein, is of particular interest in the study of lipid metabolism in man. The investigations previously reported gave rise to the following findings: (1) A moderate decline in the average serum cholesterol levels was seen in most patients on the rice diet; in no case did the levels decrease below the normal range. (2) A neutral fat lipemia, together with an increase in serum lipid phosphorus and a decreased percentage of esterified cholesterol, was observed in one-third of the patients. The decreased percentage of esterified cholesterol was associated with the development in some patients of marked sensitivity to dicumarol and of increased values for bromsulfthalein retention and thymol turbidity. This pointed to the possibility that the effects on the serum lipids were brought about through impairment of hepatic function.

The present report extends the studies to a larger number of patients and in addition presents observations during lesser degrees of restriction of dietary fat and cholesterol. Data on serum lipoprotein concentrations, as determined with the ultracentrifuge, are presented.

Several workers have correlated the serum cholesterol concentration in man with the dietary intake of cholesterol.[2–5] Within a broad range of intake levels there was no correlation with the serum total cholesterol. However, the report by Starke of studies in Kempner's clinic demonstrated that drastic restriction of both fat and cholesterol in the rice diet produced significant decreases in the free and esterified cholesterol of serum.[6]

Experimental studies in man by Keys,[7] by Hildreth[8] and by Mayer[9] and their co-workers indicated that the intake of fats, both animal and vegetable in origin, has a definite influence upon the serum cholesterol level. On the other hand Kinsell and co-workers asserted that fats of vegetable origin do not tend to maintain high serum cholesterol levels and may even have a depressant effect.[10] The weight reduction studies of Walker et al. showed that in the presence of low to moderate fat in the diet, the serum cholesterol and lipoproteins may vary in proportion to the total caloric intake.[11]

Investigations of the effects of dietary variations upon the serum neutral fat or total lipids have not been reported, except in the previous communication from this laboratory.[1] Thus the lipemia observed in certain subjects during rice diet treatment has not received further elucidation.

MATERIAL AND METHODS

The patient material for this investigation consisted of a group of hospitalized individuals with severe essential hypertension. Most of the subjects had initial resting blood pressures greater than 200/120 mm. Hg and gave evidence of impaired function of the eyes, brain,

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heart or kidneys. Patients with detectable diabetes mellitus and glomerulonephritis were excluded. The program had the following dietary phases:

Phase 1: A control period of four to eighteen weeks was conducted on a salt-poor diet of normal fat content (Diet I) served from the hospital diet kitchen.

Phase 2: The period on the control diet was followed by a period ranging from four to twenty weeks on the unmodified Kempner rice diet (Diet IV). Forty-four patients have been studied in both Phases 1 and 2.

Phase 3: Smaller groups of patients were studied on diets providing levels of fat and cholesterol intake intermediate between those of Phases 1 and 2. (*a*) Ten of the forty-four patients of Phase 2 were subsequently studied on the rice diet supplemented by various amounts of protein and fat in the form of low-sodium milk and a protein-carbohydrate powder* (Diet III). (*b*) Ten of the forty-four patients of Phase 2 subsequently received a special low-sodium diet of greater palatability than the rice diet (Diet II). (*c*) Six additional patients were studied on the special low-sodium diet (Diet II) without a preliminary period on the rice regimen. The higher fat and protein contents of this diet were provided by lonalac,* protinal® and meat.

All diets except the hospital salt-poor diet used in Phase 1 were served under strict control from a special diet kitchen.[1] The composition of the diets is described in Table I. Levels of daily fat intake of 85 gm., 55 gm., 20 to 40 gm. and 3 gm. were provided by the different regimens. Daily cholesterol intakes corresponding to the fat intake levels were approximately 1.0, 0.10, 0.03 to 0.25 and 0 gm. per day. Each of the restrictive diets (II, III, IV) was supplemented by one multiple vitamin capsule† and 0.2 gm. of ferrous sulfate daily.

Serum was analysed for free and total cholesterol, lipid phosphorus and neutral fat at weekly or bi-weekly intervals throughout the study. During the first year of the program all bloods for analysis were drawn before the patient had breakfast. It was found that on the diets under study there was no significant difference in any of the serum lipid levels between samples taken during the fasting state and after the morning meal. Therefore during the last two years the samples were taken within two hours after the patient had breakfast.

TABLE I
COMPOSITION OF DIETS

Diet	Control (Diet I)	Special Low-Sodium (Diet II)	Modified Rice (Diet III)	Rice (Diet IV)
No. of patients studied...	44	16	10	44
Calories per day.....	2220	2140	2600	2390
Sodium chloride (gm./day)..	2.5	0.25	0.25	0.25
Protein (gm./day).....	85	90	40–80	30
Carbohydrate (gm./day).	280	320	530	560
Fat (gm./day).....	85	55	20–40	3
Cholesterol (gm./day)...	1.0	0.10*	0.03–0.25*	0

* In the early part of this study the fat content of lonalac was derived entirely from milk. Five patients on Diet III received this product which provided about 0.25 gm. of cholesterol per day. More recently only one-fifth of the fat content of lonalac has been derived from milk and the remainder from coconut oil. Five patients on Diet III and all of those on Diet II received the latter product which provided 30 to 40 mg. of cholesterol daily. An additional small amount of cholesterol in Diet II was derived from the meat.

Samples for the ultracentrifugal determination of the S_f 12–20 and S_f 20–100 lipoprotein fractions were obtained after the patients had been on the specified diet for two to eighteen weeks. These samples were refrigerated and shipped by air express to Dr. J. W. Gofman who performed the analyses according to his published technic.[12,13] Only fasting bloods were used for ultracentrifugal lipoprotein analyses.

Free and total serum cholesterol were determined by the method of Schoenheimer and Sperry.[14,15] Lipid phosphorus was determined by the method of Fiske and Subbarrow[16] after extraction and wet ashing of the lipids. Conversion of lipid phosphorus to phospholipids was made using the multiplication factor of twenty-five. Total lipid was measured gravimetrically on the petroleum ether-soluble portion of an alcohol-ether extract of serum made according to the method of Bloor.[17]

It was found that the gravimetric method for total lipid suffered from errors of considerable magnitude. The weighed residue contained

* Lonalac is a low-sodium milk powder having the same protein and fat content as whole milk when diluted as directed by the manufacturer. Generous supplies were provided by the Mead Johnson & Co., Evansville, Ind. Protinal (National Drug Co.) was blended with lonalac in some instances to enrich the protein content.

† Vi-penta Perles Forte® were kindly provided by Hoffmann-La Roche, Inc., Nutley, N. J.

both inorganic salts and water. The gravimetric method was therefore compared with the manometric lipid carbon procedure of Van Slyke and Folch.[18] This comparison demonstrated that subtraction of a constant value of 200 mg. per cent from the observed total lipid levels obtained by the gravimetric method gave agreement within plus or minus 5.5 per cent on the average with the Van Slyke-Folch method. This correction has been applied to all total lipid values.

The concentration of the serum neutral fat was estimated by subtracting from the weight of total lipids the sum of the amounts of phospholipids, total cholesterol and the fatty acids of the cholesterol ester fraction (0.7 × weight of cholesterol in the esters).

Since calculation of the neutral fat content incorporates the technical errors of four different analytic procedures, no great weight can be given to the absolute values reported. However, the changes observed on the different diets are much larger than can be attributed to experimental errors.

The full effects of each new dietary regimen on the serum lipids were generally established within one week after institution of the change. Therefore the serum lipid levels reported are the averages of values observed after the first week on a given diet. The composition of the preceding diet had no significant residual influence on the lipid levels after that time.

TABLE II

SUMMARY OF CHANGES IN SERUM LIPID LEVELS DURING RESTRICTION OF DIETARY FAT AND CHOLESTEROL

Diets	No. of Patients	Intake (gm./day)		Total Cholesterol (mg. %)	Free Cholesterol (mg. %)	Lipid Phosphorus (mg. %)	Neutral Fat (mg. %)
		Fat	Cholesterol				
Control.....	16	85	1.0	219 ± 11*	64 ± 5	9.7 ± 0.5	230 ± 30
Diet II.....	16	55	0.1	207 ± 8	64 ± 5	9.6 ± 0.3	280 ± 20
Difference...	..	.....	....	−12 ± 14†	0 ± 7	−0.1 ± 0.6	+50 ± 36
Control.....	10	85	1.0	217 ± 12	61 ± 4	9.8 ± 0.5	190 ± 20
Diet III.....	10	20–40	0.03	191 ± 12	59 ± 2	10.1 ± 0.6	370 ± 50
Difference...	..	.....	....	−26 ± 17	−2 ± 4	+0.3 ± 0.8	+180‡ ± 54
Control.....	44	85	1.0	224 ± 6	64 ± 2	10.0 ± 0.3	220 ± 20
Diet IV.....	44	3	0.0	191 ± 6	61 ± 1	10.0 ± 0.4	370 ± 35
Difference...	..	.....	....	−33‡ ± 9	−3 ± 2	0.0 ± 0.5	+150‡ ± 40

Key to diets: I, control diet; II, special low sodium diet; III, modified rice diet; IV, rice diet.

* Standard error of the mean: $\sigma_{\bar{X}} = \frac{\sigma}{\sqrt{N}}$.

† Standard error of the difference: $\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$.

‡ Significant difference between control and experimental values.

RESULTS

Serum Lipid Levels on Control Period Diet. During the control period (Diet I) the hypertensive patients exhibited serum lipid levels which differed little from those of a series of healthy ambulatory subjects.* The two series were fairly matched for age and sex distribution except that there was a lower proportion of females above the age of fifty years in the healthy group than in the hypertensive series. Average serum lipid levels in fifty-one patients with hypertension were compared with single determinations of the lipid pattern in thirty-nine subjects who were leading normal lives and in

* We are indebted to Dr. Alfred Steiner for serum lipid data on the healthy subjects. These individuals showed no abnormal findings upon physical examination or in the blood count, sedimentation rate, urinalysis, electrocardiogram and chest x-ray.

TABLE III

AVERAGE SERUM LIPID LEVELS DURING PERIODS OF CONTROLLED FAT AND CHOLESTEROL INTAKE

Patient	Age and Sex	Diet	Intake (gm./day)		Total Cholesterol (mg. %)	Free Cholesterol (mg. %)	Lipid Phosphorus (mg. %)	Neutral Fat (mg. %)
			Fat	Cholesterol				
A. A.	65, M	I	85	1.0	220	58	9.4	230
		IV	3	0.0	210	76	12.8	700
A. B.	35, F	I	85	1.0	200	49	7.8	150
		IV	3	0.0	160	45	8.6	170
F. B.	58, M	I	85	1.0	200	62	11.5	270
		IV	3	0.0	250	93	15.9	1080
T. B.	56, F	I	85	1.0	240	90	14.9	490
		IV	3	0.0	300	100	14.4	550
H. C.	63, M	I	85	1.0	210	60	8.6	280
		IV	3	0.0	160	52	8.1	510
I. C.	56, F	I	85	1.0	250	68	11.3	370
		IV	3	0.0	220	73	11.0	530
J. Co.	32, F	I	85	1.0	160	46	7.8	100
		IV	3	0.0	140	39	7.3	240
R. C.	48, M	I	85	1.0	230	62	10.9	230
		IV	3	0.0	150	47	8.5	240
S. D.	52, M	I	85	1.0	190	52	8.6	120
		IV	3	0.0	170	47	8.1	200
R. E.	40, F	I	85	1.0	190	57	8.4	90
		IV	3	0.0	130	40	7.2	150
P. F.	21, F	I	85	1.0	190	52	9.3	280
		IV	3	0.0	130	44	8.3	390
A. H.	54, M	I	85	1.0	200	57	9.2	270
		IV	3	0.0	220	65	11.5	520
W. H.	60, M	I	85	1.0	260	72	10.8	....
		IV	3	0.0	200	53	8.5	....
J. I.	41, F	I	85	1.0	220	62	10.3	320
		IV	3	0.0	190	63	10.2	220
D. K.	48, F	I	85	1.0	220	61	9.8	120
		IV	3	0.0	170	47	8.1	180
E. K.	50, F	I	85	1.0	200	...	9.3	270
		IV	3	0.0	160	...	8.6	370
G. K.	48, M	I	85	1.0	240	72	10.8	360
		IV	3	0.0	190	57	9.5	290
C. L.	54, M	I	85	1.0	320	87	13.3	470
		IV	3	0.0	250	84	13.7	580
S. L.	60, M	I	85	1.0	280	80	11.2	100
		IV	3	0.0	210	61	9.3	130
A. Ma.	44, F	I	85	1.0	290	79	13.9	....
		IV	3	0.0	260	75	12.9	....
A. Mo.	42, F	I	85	1.0	240	71	10.3	110
		IV	3	0.0	170	54	9.4	260

TABLE III (*Continued*)

Patient	Age and Sex	Diet	Intake (gm./day)		Total Cholesterol (mg. %)	Free Cholesterol (mg. %)	Lipid Phosphorus (mg. %)	Neutral Fat (mg. %)
			Fat	Cholesterol				
B. M.	55, M	I	85	1.0	220	60	9.3	310
		IV	3	0.0	210	69	11.7	720
T. M.	46, M	I	85	1.0	280	84	12.7	340
		IV	3	0.0	200	60	9.6	190
M. P.	57, M	I	85	1.0	250	75	9.6	40
		IV	3	0.0	190	68	11.8	740
P. R.	61, M	I	85	1.0	170	49	8.2	140
		IV	3	0.0	130	43	7.4	170
Y. R.	44, F	I	85	1.0	290	86	12.7	320
		IV	3	0.0	250	77	12.7	250
M. Su.	67, F	I	85	1.0	220	64	10.1	170
		IV	3	0.0	240	94	13.4	720
J. W.	54, M	I	85	1.0	240	78	9.1	200
		IV	3	0.0	190	63	8.8	230
L. B.	49, M	I	85	1.0	200	52	8.2	240
		II	55	0.1	160	48	8.6	380
M. F.	40, M	I	85	1.0	200	65	8.0	330
		II	55	0.1	190	54	9.6	320
E. L.	62, F	I	85	1.0	230	70	12.4	420
		II	55	0.1	240	76	11.6	290
G. S.	47, M	I	85	1.0	330	99	15.2	530
		II	55	0.1	280	88	12.7	510
B. V.	54, F	I	85	1.0	270	71	11.2	160
		II	55	0.1	240	75	11.2	280
M. W.	55, F	I	85	1.0	250	73	11.1	250
		II	55	0.1	250	79	10.9	350
F. S.	66, M	I	85	1.0	190	52	8.0	180
		III	20–40	0.25	140	44	7.5	210
D. G.	50, M	I	85	1.0	220	66	9.4	190
		II	55	0.1	220	70	10.1	290
		IV	3	0.0	190	58	9.0	220
B. F.	51, F	I	85	1.0	210	61	8.9	170
		II	55	0.1	190	62	9.1	220
		IV	3	0.0	200	70	10.3	370
J. K.	49, M	I	85	1.0	200	58	8.7	90
		II	55	0.1	180	61	8.9	240
		IV	3	0.0	150	48	8.1	210
J. R.	48, M	I	85	1.0	240	68	9.7	190
		II	55	0.1	220	67	9.6	240
		IV	3	0.0	210	76	9.6	320
L. S.	56, M	I	85	1.0	180	61	7.8	190
		II	55	0.1	160	46	7.1	190
		IV	3	0.0	160	53	6.8	200

TABLE III (*Continued*)

Patient	Age and Sex	Diet	Intake (gm./day)		Total Cholesterol (mg. %)	Free Cholesterol (mg. %)	Lipid Phosphorus (mg. %)	Neutral Fat (mg. %)
			Fat	Cholesterol				
M. Si.	37, M	I	85	1.0	150	35	7.3	130
		II	55	0.1	210	67	9.0	260
		IV	3	0.0	160	48	7.8	110
D. W.	46, F	I	85	1.0	230	71	10.0	250
		II	55	0.1	220	66	9.3	260
		IV	3	0.0	190	63	10.2	480
J. Ce.	46, F	I	85	1.0	240	61	10.4	160
		III	20–40	0.04	270	75	12.4	220
		IV	3	0.0	230	66	11.3	360
R. G.	53, M	I	85	1.0	290	88	12.5	280
		III	20–40	0.25	150	41	7.7	170
		IV	3	0.0	190	56	9.8	240
M. K.	54, F	I	85	1.0	250	73	11.6	260
		III	20–40	0.25	200	68	11.9	700
		IV	3	0.0	180	64	11.4	440
J. N.	46, F	I	85	1.0	240	65	9.7	250
		III	20–40	0.25	220	70	12.4	550
		IV	3	0.0	210	66	12.0	580
C. R.	49, M	I	85	1.0	160	53	7.9	140
		III	20–40	0.25	160	53	9.2	330
		IV	3	0.0	150	49	9.2	310
A. W.	65, M	I	85	1.0	180	48	8.5	170
		III	20–40	0.04	200	60	10.1	370
		IV	3	0.0	160	50	8.5	150
V. B.	48, F	I	85	1.0	220	59	9.6	50
		II	55	0.1	200	58	9.4	230
		III	20–40	0.04	170	54	8.6	340
		IV	3	0.0	180	56	8.2	110
J. M.	44, M	I	85	1.0	200	58	9.0	160
		II	55	0.1	190	56	9.2	250
		III	20–40	0.04	210	64	11.4	510
		IV	3	0.0	190	72	11.6	640
S. R.	36, F	I	85	1.0	200	56	10.9	280
		II	55	0.1	180	63	8.6	210
		III	20–40	0.04	190	56	10.0	280
		IV	3	0.0	190	55	10.0	330

Key to diets: I, control diet; II, special low sodium diet; III, modified rice diet; IV, rice diet.

whom medical examination revealed no evidence of disease.

No appreciable difference existed between the two groups in average levels of free and esterified cholesterol and lipid phosphorus. The average serum neutral fat level was 46 mg. per cent higher in the patients with hypertension; this difference was statistically significant ($P = 0.05$).

Effects of Restriction of Dietary Fat and Cholesterol on Serum Lipids and Lipoproteins. The average changes in the serum lipid levels observed during restriction of dietary fat and cholesterol are summarized in Table II. The mean lipid concentrations observed upon each of the experimental diets are compared with those of the control period. The only statistically significant changes were a 15 per cent decrease in serum total cholesterol on the rice diet (Diet IV), a 95 per cent increase in serum neutral fat on the rice diet supplemented with from 20 to 40 gm. of fat

per day (Diet III) and a 68 per cent increase in serum neutral fat on the unmodified rice diet (Diet IV).

The average serum lipid levels observed in the individual hypertensive patients during the different dietary periods are given in Table III.

TABLE IV
SUMMARY OF CHANGES IN SERUM LIPOPROTEIN LEVELS DURING RESTRICTION OF DIETARY FAT AND CHOLESTEROL

Diet	No. of Patients	Intake (gm./day)		Serum Lipoproteins (mg. %)	
		Fat	Cholesterol	S_f 12–20	S_f 20–.00
Control.....	9	85	1.0	59	93
Diet II.....	9	55	0.1	66	106
Difference.....	..	..	...	+7	+13
Control.....	18	85	1.0	56	96*
Diet IV.....	18	3	0.0	65	194*
Difference.....	..	..	...	+9	+98*

Key to diets: I, control diet; II, special low sodium diet; III, modified rice diet; IV, rice diet

* Data on S_f 20–100 fraction were available in only ten patients.

It will be noted that the shift from the control to the experimental diets was followed by changes which varied greatly among the different subjects.

The changes from control levels in the average serum total cholesterol occurring on the rice diet ranged in different individuals from a decrease of 100 mg. per cent to an increase of 60 mg. per cent. Serum free cholesterol changes ranged from a decrease of 32 mg. per cent to an increase of 31 mg. per cent; lipid phosphorus from a decrease of 3.1 mg. per cent to an increase of 4.4 mg. per cent; and neutral fat from a decrease of 150 mg. per cent to an increase of 810 mg. per cent. In the smaller groups of patients studied on Diets II and III there was also little uniformity of the response in the serum lipids.

The average changes in the serum lipoprotein levels observed during restriction of dietary fat and cholesterol are summarized in Table IV. The mean lipoprotein concentrations observed upon two of the experimental diets (Diets II and IV) are compared with those of the control period. The only statistically significant change was a 100 per cent increase in the concentration of the S_f 20–100 lipoprotein fraction on the rice diet (Diet IV).

The serum lipoprotein concentrations observed in individual subjects during the different dietary periods are recorded in Table V, together with the serum lipid values obtained upon the same serum samples. As in the case of the serum lipids the response of the serum lipoproteins to the experimental diets varied greatly among different subjects.

The serum lipid and lipoprotein levels determined throughout the studies on two representative patients are illustrated in Figures 1 and 2.

Fluctuation of Serum Lipid Components. Many of the hypertensive patients showed large fluctuations of the serum lipid concentrations in the course of serial determinations. These fluctuations were not appreciably affected by restrictions of dietary fat and cholesterol. Forty-eight patients were observed for average periods of six weeks on the control diet and eight weeks on the rice diet. The average ranges of fluctuation (difference between lowest and highest values in individual patients) were as follows: total cholesterol control diet 47 mg. per cent, rice diet 46 mg. per cent; lipid phosphorus control diet 1.6 mg. per cent, rice diet 2.4 mg. per cent; neutral fat control diet 190 mg. per cent, rice diet 290 mg. per cent. Comparable information is not available in the healthy subjects.

Liver Function. Following the observation that a decreased percentage of esterified cholesterol was frequently observed in patients on the rice diet, studies of liver function were made utilizing the usual clinical tests. Although during the control period normal results were obtained in twenty-five patients, approximately 20 per cent of these patients developed abnormal bromsulfthalein retention (over 10 per cent of dye retained at forty-five minutes*), and 25 per cent abnormal thymol turbidity (over 6.0 units) while on the basic rice diet (Diet IV). The two findings usually did not occur in the same patients. Abnormal cephalin flocculation tests developed in only two patients. Abnormalities of serum bilirubin and alkaline phosphatase levels or urinary urobilinogen excretion were not observed.

Sensitivity to dicumarol was abnormally great in seven of nine patients studied on the rice diet. Prothrombin times[19] forty-eight hours after an oral dose of 300 mg. of dicumarol exceeded those of a group of hypertensive subjects who received the drug while on the control diet. (Table VI.)

COMMENTS

Patients with essential hypertension exhibit more severe arteriosclerosis than normotensive

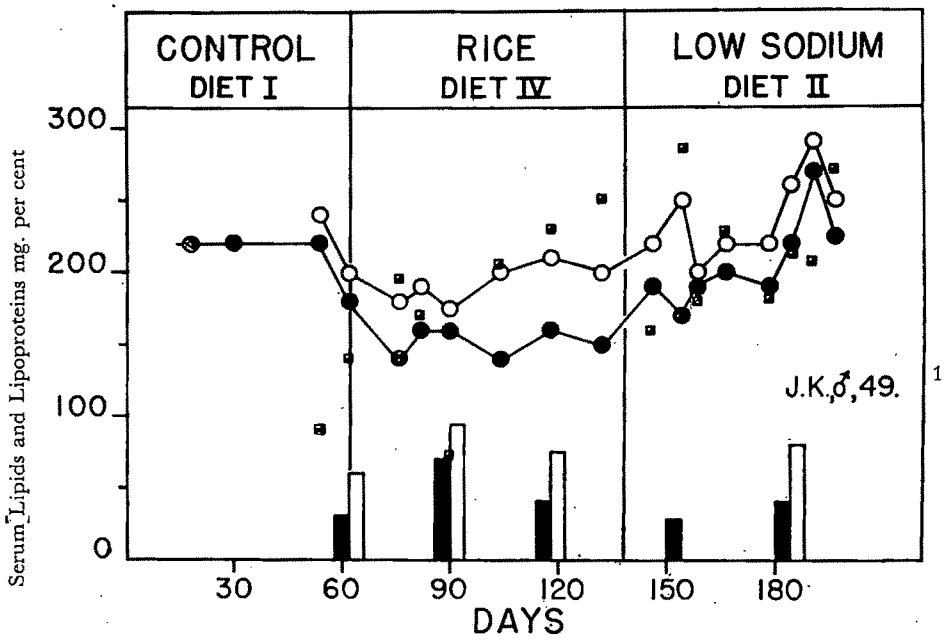
* Dose of dye 5 mg./kg. of body weight.

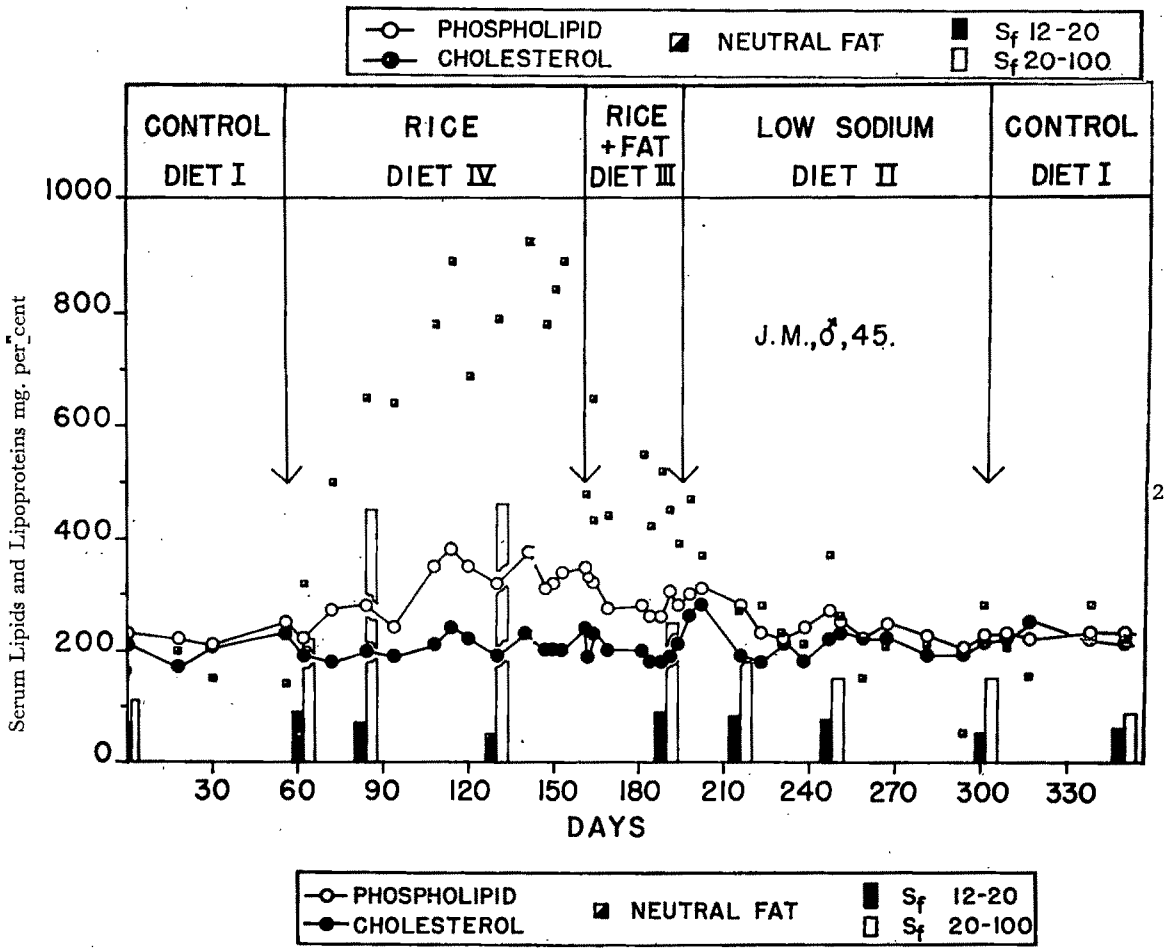
TABLE V

SERUM LIPOPROTEIN LEVELS WITH CONCOMITANT SERUM LIPID LEVELS DURING PERIODS OF CONTROLLED INTAKE OF FAT AND CHOLESTEROL

Patient	Diet No.	Time (wk.)	Intake (gm./day)		S_f 12–20 (mg. %)	S_f 20–100 (mg. %)	Total Cholesterol (mg. %)	Free Cholesterol (mg. %)	Lipid Phosphorus (mg. %)	Neutral Fat (mg. %)
			Fat	Cholesterol						
A. B.	I	6	85	1.0	40	...	222	58	8.5	110
	IV	5	3	0.0	37	...	157	43	8.6	110
T. B.	I	6	85	1.0	185	161	253	92	13.5	470
	IV	6	3	0.0	120	167	240	82	11.2	350
H. C.	I	6	85	1.0	48	...	202	54	7.8	240
	IV	6	3	0.0	33	...	143	43	7.0	300
G. D.	I	6	85	1.0	40	76	203	68	9.3	150
	II	8	55	0.1	56	131	217	60	10.6	350
	IV	7	3	0.0	45	126	199	60	9.2	270
B. F.	I	6	85	1.0	94	116	212	63	8.9	100
	II	3	55	0.1	97	95	199	60	8.6	220
	IV	2	3	0.0	107	284	220	74	11.4	470
A. H.	I	6	85	1.0	24	...	242	62	9.6	230
	IV	10	3	0.0	37	...	188	74	11.1	510
J. K.	I	6	85	1.0	33	62	182	54	8.0	140
	II	7	55	0.1	41	82	229	68	10.2	230
	IV	8	3	0.0	40	126	163	48	8.3	220
A. Ma.	I	2	85	1.0	88	...	295	81	13.1	200
	IV	18	3	0.0	143	...	230	88	14.4	620
B. M.	I	6	85	1.0	29	...	228	63	9.4	240
	IV	6	3	0.0	108	...	227	76	12.6	700
I. M.	I	6	85	1.0	59	...	238	64	10.7	130
	IV	6	3	0.0	86	...	187	52	9.1	220
J. M.	I	6	85	1.0	69	109	207	59	9.0	160
	II	8	55	0.1	75	148	233	74	9.9	250
	III	1½	60	0.1	101	226	208	60	11.1	450
	IV	4	3	0.0	66	450	198	70	12.3	650
G. P.	I	2	85	1.0	24	...	171	47	7.4	200
	IV	4	3	0.0	29	...	164	44	7.9	190
J. R.	I	6	85	1.0	69	103	237	77	10.2	330
	II	10	55	0.1	69	82	220	70	9.6	330
	IV	3	3	0.0	54	219	215	76	9.8	320
L. S.	I	2	85	1.0	41	41	160	50	7.3	190
	II	2	55	0.1	41	69	172	44	7.3	150
	IV	3	3	0.0	47	114	154	57	6.8	310
M. Si.	I	6	85	1.0	33	59	140	44	6.4	40
	II	4	55	0.1	54	58	187	52	8.5	180
	IV	5	3	0.0	55	88	162	48	7.8	180
A. W.	I	6	85	1.0	29	...	200	56	8.5	140
	IV	5	3	0.0	53	...	175	48	9.0	170
D. W.	I	6	85	1.0	52	150	215	58	10.0	340
	II	1.5	55	0.1	58	155	233	65	9.6	280
	IV	5	3	0.0	50	226	216	65	10.4	350
J. W.	I	6	85	1.0	45	80	231	72	9.4	200
	IV	7	3	0.0	62	138	262	80	8.1	50
M. W.	I	6	85	1.0	114	114	274	78	11.8	200
	II	4	55	0.1	107	137	208	78	10.3	380

Key to diets: I, control diet; II, special low sodium diet; III, modified rice diet; IV, rice diet.





FIGS. 1 and 2. Serum lipid and lipoprotein levels in two patients.

individuals of the same age and sex.[20] Dietary treatment is often recommended for these patients in the hope that either the elevated blood pressure, the arteriosclerotic process, or both will be benefited. A controlled experimental approach has been utilized in this and

TABLE VI
DICUMAROL SENSITIVITY*

	Undiluted Plasma	Diluted Plasma (1–8)
	Prothrombin Time in Seconds	
Ten patients on control diet:		
Before	19	43
48 hr.	23	47
Nine patients on rice diet:		
Before	23	52
48 hr.	42	112

* Prothrombin times before and forty-eight hours after single oral dose of 30 mg. of dicumarol.

the related investigations[1] in order to elucidate the clinical and metabolic effects of various dietary restrictions in patients with hypertension. Patients have been studied on diets with three degrees of restriction of fat and cholesterol intake.

The fat intake levels of the restricted diets ranged from 3 to 55 gm. per day and the cholesterol intake levels from 0 to 0.25 gm. per day. The salt and protein contents of the diets were additional variables. With respect to sodium chloride intake no significant changes in serum lipid pattern were produced by the addition of 0.5 to 3 gm. of sodium chloride per day to the low sodium regimens (Diets II, IIC or IV) of five patients. The influence of variations in protein intake has not been fully assessed. No effect on the serum lipid levels was produced by raising the protein content of the supplemented rice diet (Diet III) to 40 and to 80 gm. per day. The rice diet (IV) is unusual in the very character of its foods since it contains only rice, fruits and fruit juice and sugar. Therefore it is possible that some unrecognized deficiency contributed to the results obtained with this diet.

Partial or nearly complete restriction of dietary fat and cholesterol under the conditions of these studies failed to reduce serum lipid and lipoprotein levels in any patient below the normal range of values. The question is raised as to how these serum concentrations are maintained in the absence of direct dietary contributions of fatty acids and cholesterol.

Unless a starvation regimen be imposed the restriction of dietary fat implies an increase in the carbohydrate intake. Chaikoff[21] and Stetten[22] and their co-workers have shown in experiments with rats that a large proportion (perhaps one-third) of ingested glucose is transformed into fat before utilization. This transformation, which is at least partly under the control of insulin, is carried on actively at normal and high levels of carbohydrate intake and regardless of the absence of fat or protein from the diet.

Since the storage capacity of the body for carbohydrate is limited, conversion of carbohydrate into fat is to be expected when food intake exceeds energy requirements. Evidence for such a conversion is available in our studies. A comparison of changes in total body water (measured by means of antipyrine*) with changes in body weight occurring on the rice diet (IV) revealed that in eleven of the seventeen patients studied there was a greater loss of water than of weight. The magnitude of these increases ranged from 1 to 10 kg. over periods of three to fifteen weeks on the rice diet. This paradox presumably resulted in part from increases in the body fat. Confirmatory evidence is provided by the finding of a postabsorptive respiratory quotient* slightly greater than 1.0 in one subject who was gaining weight while losing body water. Such a respiratory quotient is generally presumed to indicate the transformation of carbohydrate into fat.

The continuation of normal serum cholesterol concentrations after elimination of the dietary cholesterol intake is probably also due to an active mechanism for endogenous synthesis. Abundant supplies of the known precursors of cholesterol are available from the metabolic pools of small organic molecules in the body. Abell and Kendall[23] in this laboratory have shown that the excretion of cholesterol in the feces is not greatly reduced in patients on the

* For these measurements we are indebted to the New York University Research Service, Goldwater Memorial Hospital. Drs. M. Dunning and E. Y. Berger performed the studies of total body water and Dr. Morton Galdston determined respiratory quotients in selected patients.

rice diet. (Table VII.) Four patients excreted amounts (0.3 to 0.9 gm. per day) of total digitonin-precipitable sterols ranging from 50 to 125 per cent of their excretion on the control diet. The average change on the restricted diet was a decrease of only 20 per cent in fecal sterol

TABLE VII
FECAL STEROL EXCRETION DURING CONTROL AND RICE DIETS

Patient	Weeks on Rice Diet	Total Digitonin-precipitable Sterols (mg./day)		
		Control (I)	Rice (IV)	Change (%)
F. S.	28	430	300	−30
B. M.	6	480	600	+25
H. C.	6	1150	920	−20
A. W.	11	730	350	−53
	Average	700	540	−23

excretion despite the fact that dietary sterol intake decreased from 1 gm. per day on the control diet[1] to 0.1 gm. per day of plant sterols (digitonin-precipitable) on the rice diet (IV). This study provides strong evidence of an active synthesis and excretion of cholesterol in the body when the exogenous supply of preformed cholesterol is cut off.

There was a marked variation in the response of different individuals to the dietary restrictions. The changes in concentration of the serum lipids and lipoproteins following restriction of dietary fat and cholesterol cannot be predicted for any given patient. The type of change observed cannot be correlated with the degree of elevation of the blood pressure, with the response of the blood pressure to treatment, with the presence or absence of overt arteriosclerosis, or with changes in body weight.

In these studies changes in cholesterol intake did not have any effect distinct from that resulting from fat restriction. The special low sodium diet (Diet II) which provided 55 gm. of fat but *only 40 mg. of cholesterol* daily did not significantly change the serum lipid or lipoprotein levels from the values observed on the control diet (Diet I), which contained 85 gm. of fat and 1 gm. of cholesterol daily. Our observations indicate that it is the restriction of dietary fat rather than cholesterol which affects the metabolism of the serum cholesterol and other lipids. This finding is in agreement with the results of Keys[24] and of Hildreth et al.[25]

It has been shown that patients on the rice diet frequently develop abnormal responses to some of the tests used to evaluate liver function. In a previous paper[1] these abnormalities were considered to be indicative of a mild disorder of liver function which might also be responsible for the changes in the serum lipid pattern observed during restriction of dietary fat. This point of view has now been modified. With one exception the abnormal results in thymol turbidity tests occurred only in patients who exhibited neutral fat lipemia on the rice diet. We have observed that transient elevation of the thymol turbidity test also occurs during alimentary lipemia. Kunkel and Hoagland[26] have reported that false-positive thymol turbidity tests are associated with the lipemia occurring in the nephrotic syndrome. Thus it seems probable that the elevations of thymol turbidity observed in this study are related to changes in the serum lipoproteins involved in the reaction with thymol rather than to disordered function of the liver.

The hypersensitivity to dicumarol noted in this investigation was probably due to faulty absorption of vitamin K occasioned by decreased absorption of fat on the restricted diet. The hypersensitivity could be abolished either by adding fat to the diet or by administering 20 mg. per day of a water-soluble preparation of vitamin K.

The decline in the percentage of cholesterol in the ester fraction and the occasional finding of increased bromsulfthalein retention thus remain as the only indicators of hepatic dysfunction in patients on the rice diet. Although altered metabolic processes in the liver probably contribute to the observed changes in serum lipid and lipoprotein patterns, there is no cogent argument for clinically important liver damage.

SUMMARY AND CONCLUSIONS

Controlled observations of serum lipid and lipoprotein levels have been made in a series of hospitalized patients with severe essential hypertension undergoing both partial (twenty-three patients) and practically complete (forty-four patients) restriction of dietary fat and cholesterol. There is no information to indicate that the observations reported here are peculiar to the hypertensive individual.

Three major findings resulted from this investigation: (1) The restriction of dietary fat

to 3 gm. per day and elimination of cholesterol intake did not reduce the serum lipid or lipoprotein levels in any patient below the normal range of values. Compensatory metabolic mechanisms exist for maintaining the serum lipid and lipoprotein concentrations when lipid is not available in the diet. Carbohydrate and protein appear to be ready sources for endogenous synthesis of the lipids present in the serum. (2) The results of drastic dietary restriction of fat and cholesterol showed great variability among patients in the effects on the serum lipid patterns. Serum cholesterol ester levels usually declined with restriction of daily fat intake below 40 gm. Free cholesterol and lipid phosphorus concentrations showed no consistent change. However, one-fifth of the patients undergoing such restriction exhibited a lipemia chiefly marked by elevation of the serum neutral fat. Data on serum lipoproteins showed that the dietary restriction did not significantly alter the concentration of the S_f 12–20 class of particles and that substantial increases in the S_f 20–100 class were provoked. The response of the serum lipids and lipoproteins to restriction of dietary fat was not predictable for any given patient. The type of change observed was not correlated with the severity of the hypertension or with the presence or absence of arteriosclerotic complications. (3) The restriction of dietary fat rather than the restriction of dietary cholesterol is responsible for the observed alterations of the serum concentrations of cholesterol and other lipids.

Acknowledgments: The authors wish to express their appreciation to Drs. D. M. Watkin, H. F. Froeb, G. H. Eurman and the members of the resident staff of the Research Service for assistance in the conduct of the clinical program; to Miss Hannah Epstein, dietitian; to Mr. W. Meyer for the illustrations and to the following for technical assistance, Misses C. Meenan, S. English, C. Zomzely, G. McPhee and Z. Fox, Mrs. S. Yamaguchi, and Messrs. L. Ellenbogen, F. Nierman and R. Carter.

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The Effects of Sitosterol on Serum Lipids*

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IN 1951 Peterson reported that the addition of mixed soybean sterols to a cholesterol-enriched diet fed to chicks prevented the hypercholesterolemia which otherwise occurs on a high cholesterol diet.[1] On the basis of this observation he proposed that the absorption of cholesterol is interfered with by one or more of the constituents of soybean sterol mixtures, most likely sitosterol. That sitosterol might interfere with reabsorption of cholesterol secreted into the intestinal tract in mice had previously been suggested by Sperry and Bergmann.[2]

Stimulated by Peterson's report and the growing body of evidence linking cholesterol with atherosclerosis[3] a number of investigators have been exploring the effects in man of the administration of soy sterols, with conflicting results reported.[4,5] Unreported studies in this laboratory noted a hypocholesterolemic effect in man of a soy sterol mixture containing approximately 80 per cent gamma-sitosterol, the greater part of the remainder being stigmasterol.

In a preliminary report[6] we summarized the effects of the administration of beta-sitosterol (a stereoisomer of the gamma-sterol) up to eighteen weeks on nine patients on an unrestricted diet. The sterol used was derived from tall oil or cottonseed oil and contained 98 per cent beta-sitosterol. In all patients there was a reduction in serum total cholesterol, the mean fall ranging from 6.7 to 20.0 per cent of the control level. A reduction in the ratio of total cholesterol to lipid phosphorus was also noted.

Sufficient data have now been obtained in fourteen patients to permit evaluation of the effects of the prolonged administration of beta-sitosterol on serum cholesterol and other lipid fractions.

METHODS

Fourteen subjects (Table 1) were studied for periods ranging from thirteen to sixty-four weeks, the mean period of observation being forty weeks. Twelve subjects had elevated serum cholesterol levels not associated with hypothyroidism, diabetes mellitus or the nephrotic state. The remaining two volunteers were "normal." A major factor in the selection was the ability and willingness of the subjects to cooperate and adhere to the prolonged schedule of treatment.

Six to 8 gm. of beta-sitosterol† was administered orally immediately before the ingestion of food, the usual total being 20 to 25 gm. per day. As much as 50 gm. per day has been administered on occasion. A placebo preparation, similar in appearance, taste and consistency, was administered for one or more periods to each patient. At no time did the patient know whether he was taking sitosterol or a placebo and, with the exception of the three faculty members included in the study (H-1, H-2, H-4), none was aware that a placebo was used. This precaution was taken to avoid possible influence of such knowledge on the patient's selection of diet, particularly with regard to fat and cholesterol content. There was no restriction as to the amount or type of diet.

Each patient was seen at one or two week intervals, his weight recorded, and a fasting specimen of blood drawn at approximately 9 A.M. The blood was allowed to clot and the serum separated by centrifugation. If there was any evidence of hemolysis the specimen was discarded. Total cholesterol was determined by the method of Abell,[7] lipid phosphorus by the method of Zilversmit,[8] and total lipid by the method of de la Huerga.[9] Multiplication of lipid phosphorus by 25 permits its expression as phospholipid. That portion of the total lipid not

† Supplied by Eli Lilly and Company, Indianapolis, Ind.

* From Department of Medicine and Institute for Medical Research, University of Louisville School of Medicine, and the Louisville General Hospital. This work was supported by grants from Eli Lilly and Company, Indianapolis, Indiana, and the Commonwealth of Kentucky Medical Research Commission. Presented in part at the meeting of the American Society for the Study of Arteriosclerosis, 1954 Chicago, Ill.

accounted for as phospholipid or cholesterol was designated neutral fat.[10]

In seven subjects the effect of sitosterol on serum lipoproteins, as determined by ultracentrifuge, was studied. At intervals during both control and treatment periods a sufficient amount of blood was collected from these subjects to permit this additional analysis. The portion of the serum specimen for ultracentrifugal analysis was refrigerated and shipped air mail to Indianapolis. Analysis was performed at a solution density adjusted to 1.063, and a template employed to facilitate the calculations.[11]

TABLE I

CLINICAL DATA OF SUBJECTS TREATED WITH SITOSTEROL

Patient	Age and Sex	Observation (weeks)	Sitosterol Therapy (weeks)	Remarks
A-6	43, M	34	28	Xanthoma tuberosum (confirmed by biopsy)
A-5	43, M	44	36	Acute myocardial infarction six weeks prior to treatment; angina pectoris
A-4	41, M	48	40	Acute myocardial infarction one year prior to treatment; angina pectoris
H-7	77, F	56	52	Acute myocardial infarction three years prior to treatment; angina pectoris
A-3	51, F	28	20	Xanthelasma
H-6	50, M	56	32	Acute myocardial infarction nine years prior to treatment; obesity
H-8	40, F	64	52	Undiagnosed disease one year prior to treatment (hypertension, anasarca, proteinuria, abnormal pattern of serum proteins by electrophoresis and acute arteritis by biopsy; duration four months)
H-5	33, M	60	52	Labile hypertension
A-2	41, M	22	16	Acute myocardial infarction eighteen months prior to treatment; xanthelasma
H-4	58, M	62	56	Peripheral arterial insufficiency
H-3	65, M	13	8	Acute myocardial infarctions one, four and five years prior to treatment; angina decubitus
A-1	34, F	34	28	Idiopathic hyperlipemia
H-2	43, M	24	8	Obesity; otherwise "normal"
H-1	46, M	22	8	"Normal"

RESULTS

Changes in serum lipids consequent to sitosterol administration in a representative patient (H-6) are shown in Figure 1. During each of the three periods of sitosterol administration serum total cholesterol and total lipid were maintained at levels significantly below the mean control values. The substitution of a placebo preparation during the first two control periods was accompanied by a return to the pretreatment range. During the final control period the patient received neither sitosterol nor a placebo, and again serum lipids returned to the pretreatment range. The lack of consistent effect on lipid phosphorus in this patient is considered representative; most other patients, however, demonstrated a more consistent fall in neutral fat during sitosterol administration.

The effects of sitosterol administration on the various serum lipids in the fourteen patients

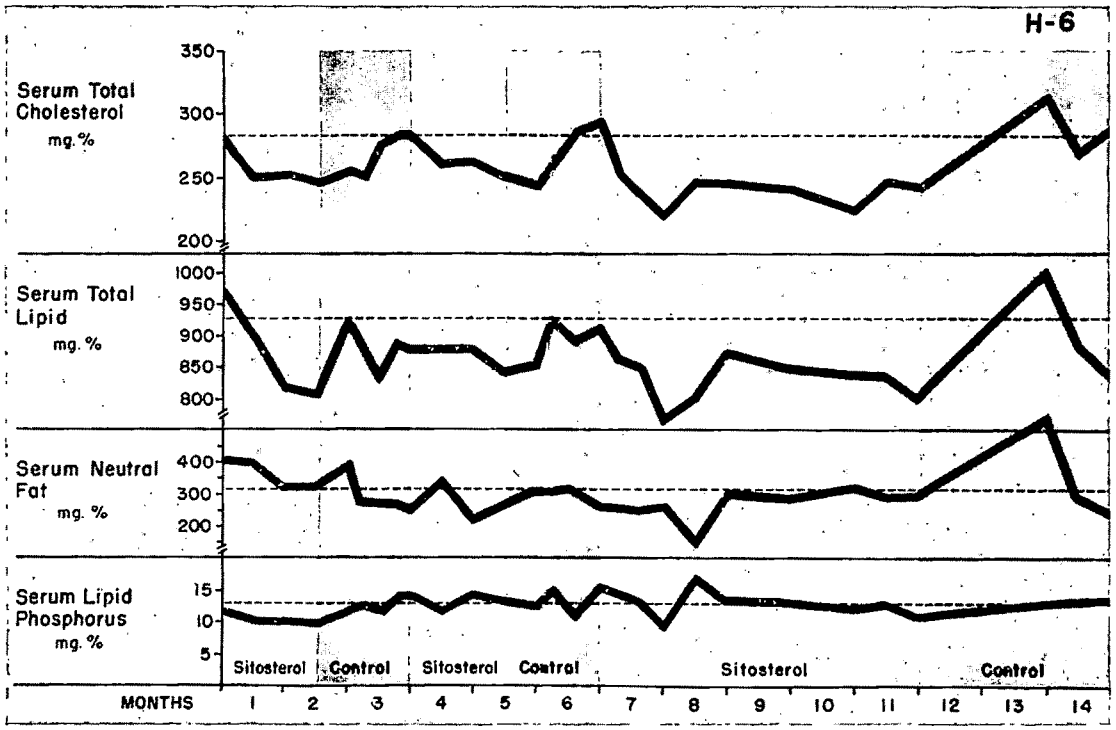


FIG. 1. Effects of repeated administration of sitosterol; effects of sitosterol on the various serum lipids of a representative hypercholesterolemic patient. Broken lines indicate mean control.

studied are presented in composite dot diagrams (Figs. 2, 3, 4, 5 and 6) constructed in a similar manner. The per cent deviation from mean control values is represented on the vertical axis. At the left each dot represents one control determination, expressed as per cent deviation from the mean control value for that subject. To the right of the broad vertical line the data are expressed in terms of months, each dot representing the mean of the two to four determinations made during the month. The mean control level is represented by the broken line; the solid line represents the mean effect of sitosterol administration. The two patients (A-1 and A-6) with pronounced hyperlipemia displayed unusual spontaneous fluctuations in serum lipids and are designated by triangles.

The administration of beta-sitosterol is accompanied by a consistent fall in serum total cholesterol. (Fig. 2.) This effect is manifested by the end of one week, and a further decline occurs during the succeeding several weeks. The effectiveness of sitosterol in lowering serum cholesterol is sustained throughout the period of study, there being no indication of a tendency to "escape."

The degree, approximately 20 per cent, and consistency of the reduction in serum total lipid (Fig. 3) are similar to those of cholesterol. Since serum cholesterol constitutes only about one-third of the total lipid, it is evident that one or more other serum lipids must be decreased following administration of sitosterol.

A less pronounced and less consistent effect was observed on serum lipid phosphorus. (Fig. 4.) The variability of the lipid phosphorus during both control and treatment periods can be seen to be appreciably greater than that of either total cholesterol or total lipid.

The remainder of the total lipid not accounted for as phospholipid or cholesterol has been designated neutral fat. The mean reduction in neutral fat (Fig. 5) is in general comparable to that in cholesterol. An even greater degree of variability of neutral fat is apparent.

The ratio of serum total cholesterol to lipid phosphorus has been described as being relatively constant.[12] The variability in control values of this ratio (Fig. 6) is therefore somewhat unexpected. Some reduction of the total cholesterol/lipid phosphorus ratio resulted from sitosterol administration, a reflection of the greater reduction of cholesterol as compared to that of lipid phosphorus.

The mean levels of each of the various serum

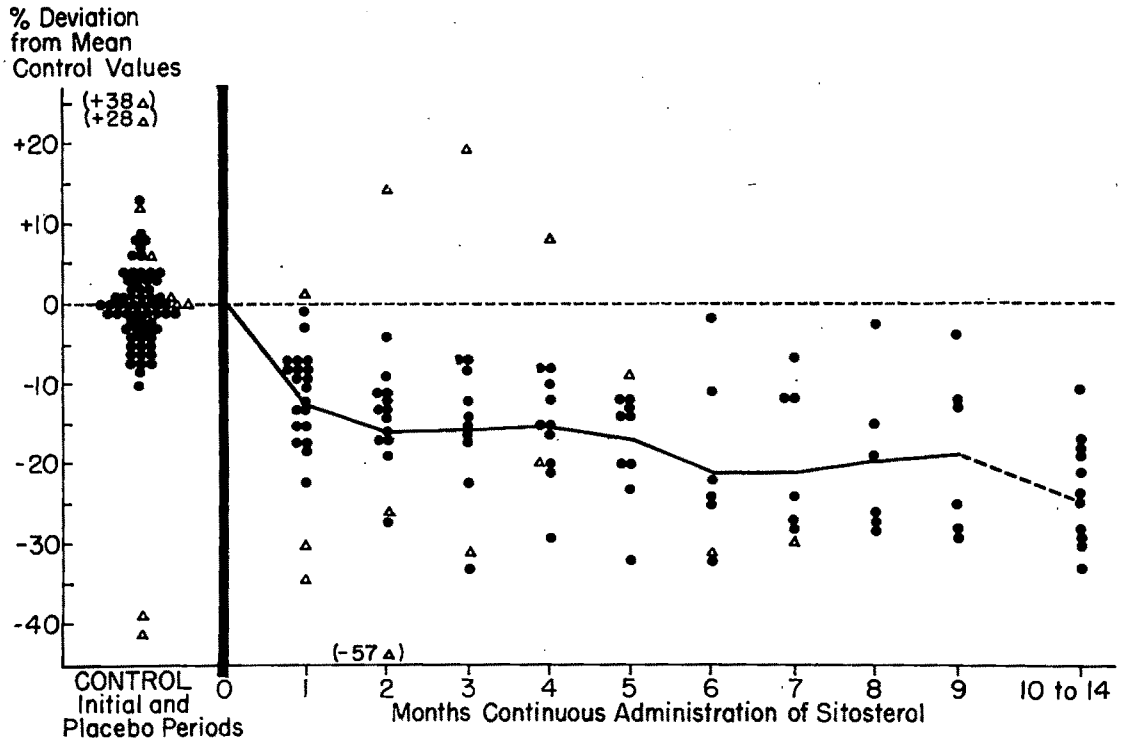


FIG. 2. Per cent change; serum total cholesterol (fourteen subjects). Effect of sitosterol on serum total cholesterol. The triangles designate values in two patients (A-1 and A-2) with pronounced hyperlipemia (see text).

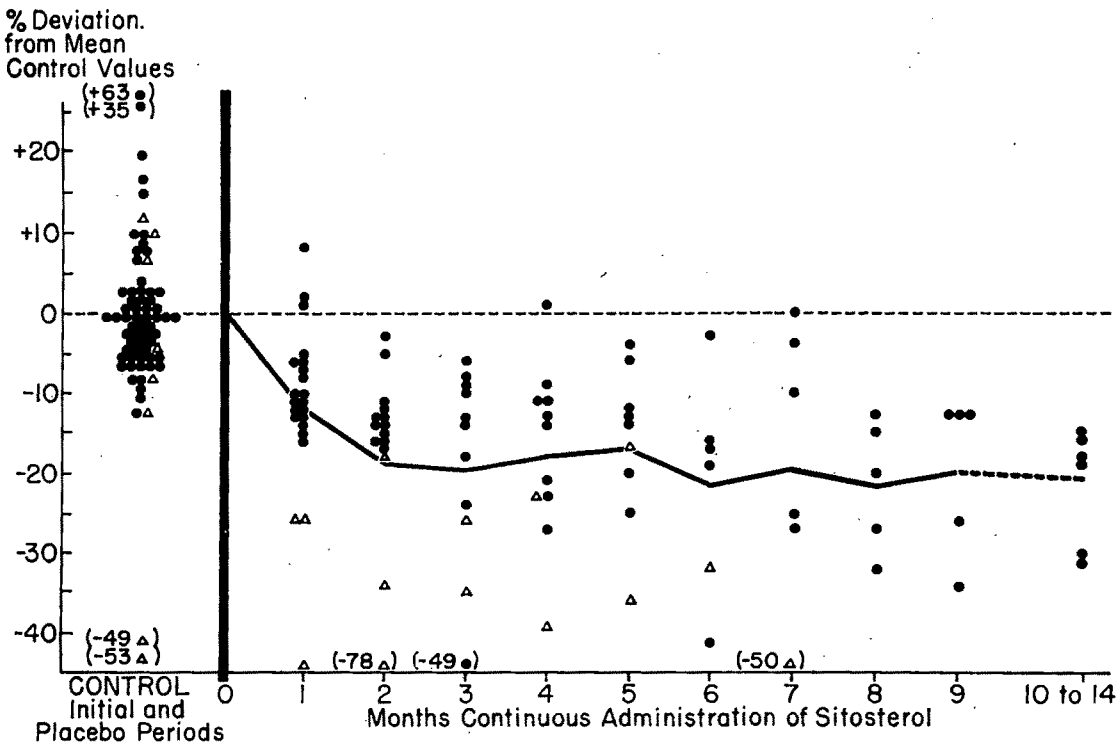


FIG. 3. Per cent change; serum total lipid (thirteen subjects). Effect of sitosterol on serum total lipid.

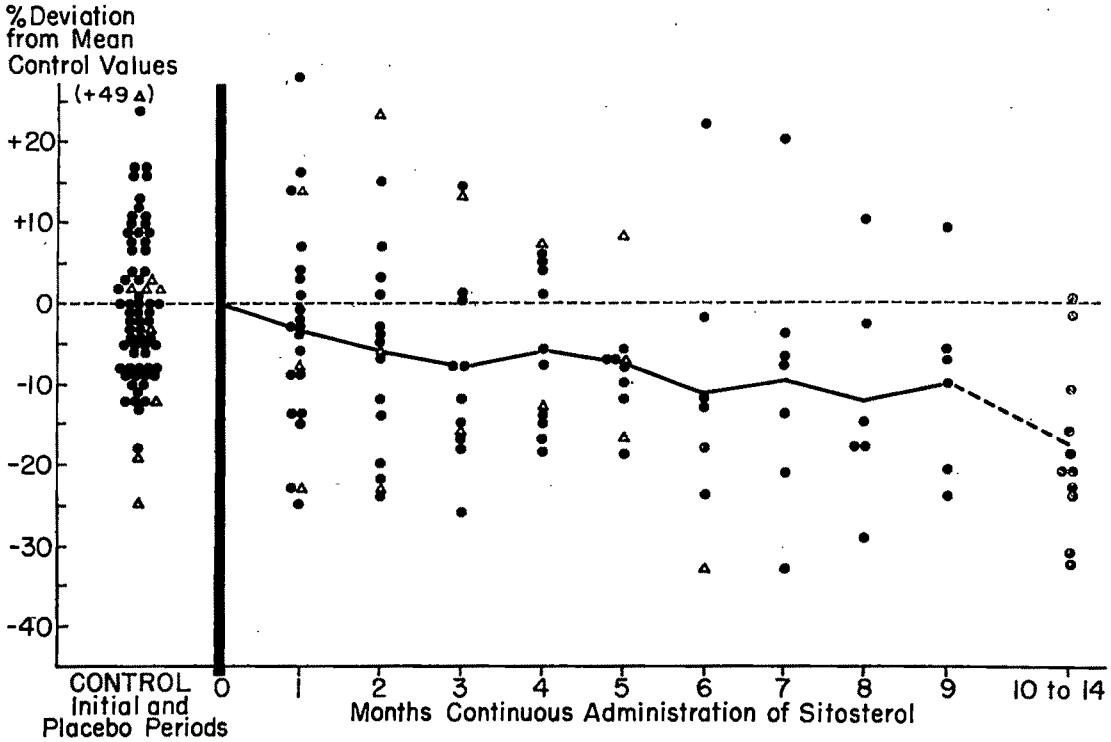


FIG. 4. Per cent change; serum lipid phosphorus (fourteen subjects). Effect of sitosterol on serum lipid phosphorus.

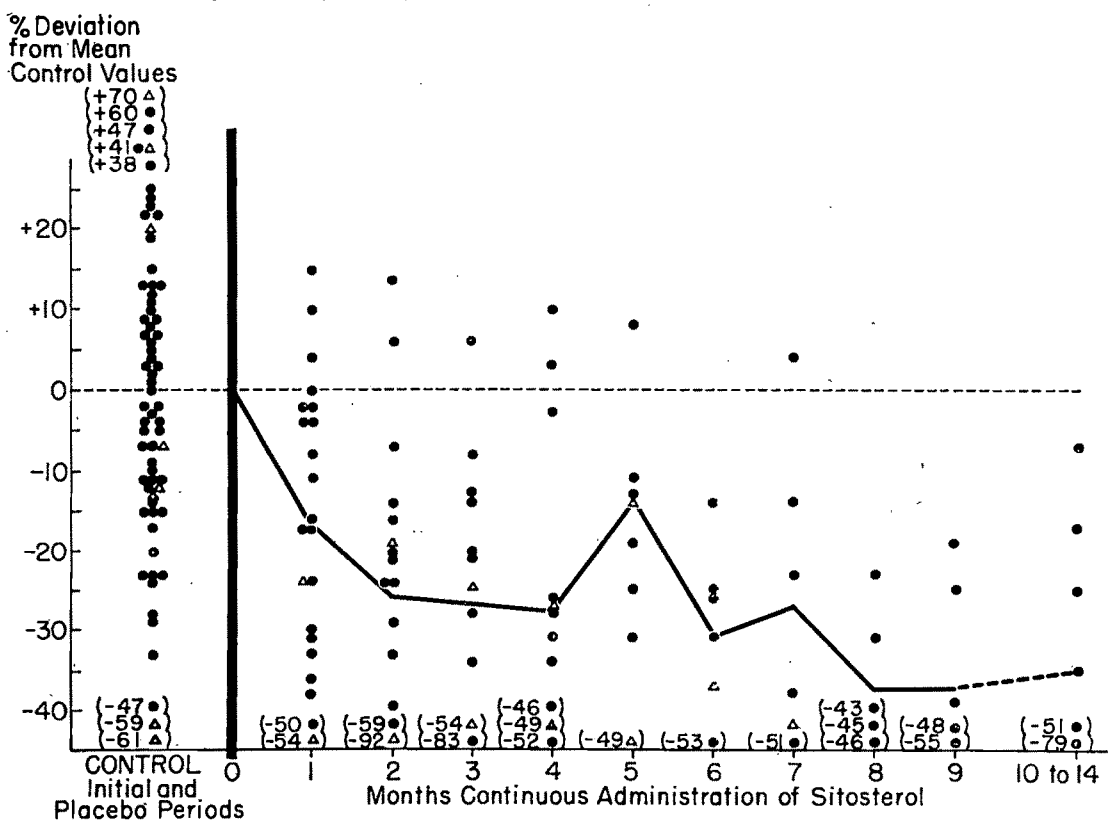


FIG. 5. Per cent change; serum neutral fat (thirteen subjects). Effect of sitosterol on serum neutral fat.

lipids during control periods and periods of sitosterol administration are tabulated in Table II. All determinations of serum lipids are included in the data presented except those made during the two-week period immediately following discontinuation of sitosterol. These values

The two patients with pronounced hyperlipemia, designated by triangles in Figures 2 to 6, deserve special comment. One subject, A-6, displayed marked elevation of all the lipid fractions determined, with a mean control serum total cholesterol of 843 mg./100 ml. The other

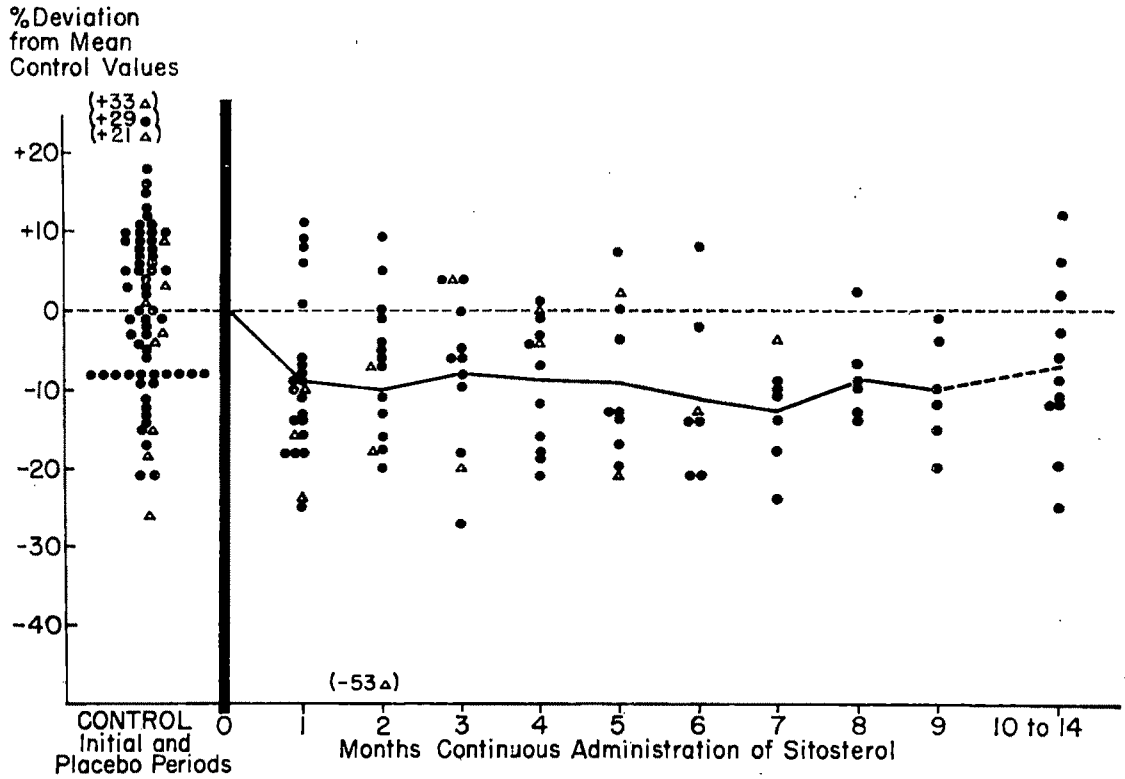


FIG. 6. Per cent change; ratio serum total cholesterol/lipid phosphorus. Effect of sitosterol on serum total cholesterol/lipid phosphorus ratio.

were omitted in order to minimize any possible persistent sitosterol effects on the control values. Data on serum total lipid and neutral fat are missing for subject H-8, whose serum displayed a peculiar clotting tendency that made routine determination of total lipid impractical.

The subjects are listed in order of decreasing control level of serum cholesterol. It can be seen that all patients displayed a fall in cholesterol; the fall ranged from 28.8 per cent of the control level in patient A-5 to 6.5 per cent of the control value in subject H-2. It will be observed that in general the higher the control level of cholesterol the greater the fall during sitosterol administration, not only in absolute values but in terms of per cent as well. Thus the twelve hypercholesterolemic patients experienced a mean fall of 16.0 per cent while the two "normal" subjects had a mean fall of 6.8 per cent.

subject, A-1, displayed an increase chiefly in neutral fat, her mean control cholesterol being 224 mg./100 ml. In both there were wide fluctuations in serum lipid levels during both control and treatment periods (Table II). Although the mean fall in cholesterol and other lipids displayed by these two subjects is comparable to that of the other patients, the wide fluctuations make interpretation difficult and more observations will be needed to evaluate fully the effects of sitosterol in such hyperlipemic subjects.

The mean levels of serum lipoproteins of the S_f classes determined during control periods and periods of sitosterol administration in seven patients are shown in Table III. Although a trend toward lower levels of S_f 3–100 lipoproteins during the administration of sitosterol is apparent, this effect is much less consistent than that on serum cholesterol and total lipid.

COMMENTS

A sustained lowering of serum total cholesterol results from the prolonged administration of beta-sitosterol. A concomitant reduction in serum total lipid, neutral fat and, to a lesser degree, lipid phosphorus occurs. The employment of a placebo during control periods and the failure of any of the patients to lose weight during the period of study, would seem to eliminate any possibility that the effects observed were due to dietary modification. The number of determinations performed in each patient allows for the recognized variability in serum cholesterol.[13]

Studies with animals have done much to explain the probable mechanism of the hypo-

TABLE II

EFFECTS OF SITOSTEROL ON SERUM LIPIDS

(Expressed in mg./100 ml.)

Patient		Total Cholesterol		Lipid Phosphorus		Neutral Fat		Total Lipid		TC/LP Ratio	
		Control	Sitosterol	Control	Sitosterol	Control	Sitosterol	Control	Sitosterol	Control	Sitosterol
A-6	Mean	843	614	33.5	28.1	3092	2264	4772	3581	24.1	21.8
	S.D.	56	84	0.9	2.9	542	290	500	413	0.9	1.9
A-5	Mean	361	257	17.5	13.9	457	283	1254	889	20.4	18.7
	S.D.	26	12	1.6	1.2	74	106	123	138	1.1	1.2
A-4	Mean	330	281	14.7	13.5	349	278	1019	899	22.5	20.9
	S.D.	19	15	1.3	0.1	52	46	73	34	1.2	1.9
H-7	Mean	297	237	14.8	13.3	269	177	937	743	20.3	18.0
	S.D.	13	14	1.7	2.5	54	60	26	56	2.9	1.4
A-3	Mean	286	253	16.1	14.3	213	193	902	804	17.7	17.7
	S.D.	17	11	1.3	1.9	38	57	75	36	1.3	1.7
H-6	Mean	285	244	12.8	12.3	314	290	927	839	22.6	20.6
	S.D.	13	11	1.2	1.4	106	43	103	22	2.6	2.1
H-8	Mean	276	214	15.1	12.1	....	....	....	....	18.4	17.6
	S.D.	4	27	1.2	1.1	....	....	....	....	1.8	1.5
H-5	Mean	273	235	14.7	13.6	412	403	1054	965	19.0	17.6
	S.D.	10	11	2.2	1.5	47	48	73	80	3.6	1.8
A-2	Mean	264	243	12.6	13.0	208	180	788	749	21.1	18.9
	S.D.	7	11	1.5	0.4	38	41	24	31	2.1	1.1
H-4	Mean	261	233	12.1	12.9	338	235	902	790	21.8	18.4
	S.D.	16	17	1.6	1.3	86	34	64	52	2.7	1.7
H-3	Mean	260	229	12.2	11.4	357	298	920	813	21.5	20.3
	S.D.	11	14	1.1	5.0	57	1	43	3	2.2	1.8
A-1	Mean	224	206	12.6	13.1	1520	664	2059	1198	17.8	15.2
	S.D.	68	43	3.1	1.9	739	259	870	344	4.0	2.9
H-2	Mean	215	201	11.4	13.8	291	212	791	758	18.9	14.9
	S.D.	10	9	1.0	1.1	42	94	37	131	1.5	0.9
H-1	Mean	196	182	10.7	10.2	290	240	752	688	18.6	17.9
	S.D.	11	7	1.3	1.4	73	67	57	23	2.2	3.2

cholesterolemic effect of sitosterol. The suggestion that sitosterol acts by interfering with the absorption of cholesterol from the digestive tract[1,2] has been confirmed by Hernandez using the technic of thoracic duct cannulization in rats.[14,15] He fed C^{14}-labeled cholesterol, alone and in conjunction with either beta-sitosterol or mixed soy sterols, and observed that addition of the plant sterols resulted in a pronounced decrease in the C^{14} recovered in the thoracic duct lymph.

TABLE III
EFFECT OF SITOSTEROL ON SERUM LIPOPROTEINS

Patient	Sf 3–10 (gm./100 ml.)		Sf 10–30 (gm./100 ml.)		Sf 30–100 (gm./100 ml.)		Total Cholesterol (mg./100 ml.)	
	Control	Sitosterol	Control	Sitosterol	Control	Sitosterol	Control	Sitosterol
A-5	.39	.34	.17	.08	.37	.12	357	272
A-4	.46	.28	.06	.05	.09	.06	360	266
H-7	.21	.31	.07	.03	.01	.01	282	234
A-3	.70	.28	.12	.03	.09	.12	298	265
H-6	.43	.37	.03	.10	.08	.07	304	253
A-2	.27	.25	.02	.07	.01	.01	270	238
H-4	.44	.29	.10	.05	.12	.07	261	241

The structural formulas of cholesterol and beta-sitosterol are quite similar, differing only in the presence of an ethyl group on carbon-24 of sitosterol. Both possess a free hydroxyl group on carbon-3. It is by means of this carbon-3 hydroxyl group that cholesterol, in the presence of bile salts and pancreatic esterase, combines with fatty acids to form cholesterol esters. That soy sterols under the same conditions also form esters has recently been shown by Swell and coworkers.[16]

Esterification of the greater part if not all of the cholesterol absorbed occurs at some point between its introduction into the gut and its arrival in the lymph. Peterson has observed that when cholesterol is fed as an ester its rate of absorption is decreased rather than increased.[17] This observation is best understood if esterification is considered as a step in the transport mechanism by which cholesterol is absorbed. Thus prior esterification would be expected to impede rather than to facilitate absorption.

In view of these considerations it is suggested that sitosterol interferes with cholesterol absorption by competing for esterification, a step in the transport mechanism by which cholesterol is absorbed. Schoenheimer failed to demonstrate by chemical methods any absorption of sitosterol, and it was long considered to be essentially non-absorbable.[18] Recently Gould, employing tritium-labeled sterols, has restudied the absorption of cholesterol and sitosterol.[19] He observed that beta-sitosterol was absorbed, though much less readily than cholesterol. Sitosterol also disappeared from the blood much more rapidly than cholesterol and did not accumulate in the body. The demonstration that sitosterol is absorbed, though poorly, perhaps lends support to the concept that it acts by competing with cholesterol for absorption.

It seems probable that sitosterol interferes with the absorption of the cholesterol excreted in the bile as well as that present in the diet since both are introduced into the intestine in the free state. That the results of sitosterol administration are not identical with those of restriction of dietary cholesterol (a measure that does not consistently lower serum cholesterol unless accompanied by restriction of dietary fat[20]) is most likely due to its effect on biliary cholesterol.

The administration of sitosterol is accompanied by a significant lowering of serum neutral fat. This is presumably a secondary effect to the lowering of serum total cholesterol, an appreciable part of which is present in complex combinations also containing neutral fat. The possibility remains, however, that sitosterol in the dosage administered might interfere also with fat absorption, leading to lower levels of serum neutral fat and contributing to the reduction of serum cholesterol. Results of current studies of the effect of sitosterol on fat absorption may serve to clarify the mechanism by which sitosterol lowers serum neutral fat.

Gofman and coworkers have been concerned with the physicochemical state of the serum lipids as determined by the analytical ultracentrifuge and as expressed in Svedberg units of migration (S_f). They report that restriction of dietary fat results in a reduction of the serum levels of S_f 12–100 lipoproteins, classes which they consider to be particularly concerned with atherosclerosis.[21] The administration of sitosterol also appears to effect a lowering of these classes of lipoproteins.

The amount of sitosterol employed in these studies, 20 to 25 gm. per day, was largely empiric. As little as 10 gm. per day has been noted to have a hypocholesterolemic effect; higher dos-

age, up to 50 gm. per day, appeared to have a more pronounced effect, but optimal dosage has not yet been determined.

Attempts to lower or otherwise alter the serum lipids and lipoproteins have been the natural outgrowth of the cholesterol concept of atherogenesis. The changes in serum lipids and lipoproteins resulting from sitosterol administration, on theoretical grounds, may be considered desirable in atherosclerotic states. The absence of any toxic or adverse side effects of sitosterol permits continuation of these studies to evaluate its effect on atherosclerosis in man.

SUMMARY

1. The effects of the plant sterol, beta-sitosterol, on serum cholesterol, other lipids and lipoproteins have been studied. Twenty to 25 gm. of sitosterol per day was administered for prolonged periods to fourteen patients on free diets.

2. A sustained lowering of serum total cholesterol results from the prolonged administration of sitosterol. A concomitant reduction in serum total lipid, neutral fat and, to a lesser degree, lipid phosphorus occurs.

3. A trend toward lower levels of S_f 3–10, 10–30 and 30–100 classes of lipoproteins occurs during sitosterol administration but this effect is less consistent than the reduction of serum cholesterol.

4. The effects of sitosterol are attributable to its interference with absorption of cholesterol. They are not identical with those of dietary cholesterol restriction, since sitosterol would seem to reduce absorption of cholesterol present in the bile as well as that present in the diet.

5. It is suggested that sitosterol interferes with cholesterol absorption by competing for esterification, a step in the transport mechanism by which cholesterol is absorbed.

6. The changes in serum lipids and lipoproteins resulting from sitosterol administration are in the direction generally considered desirable in atherosclerosis, and the absence of any toxic or adverse side effects permits its further study in man.

Acknowledgments: The authors are indebted to Dr. Robert E. Shipley, Dr. Edwin O. Davisson and Harold W. Fisher, Lilly Research Laboratories, Indianapolis, Ind., for the lipoprotein determinations. Frank W. Shook, Jr. and Catherine Bauscher, Department of Visual Education, University of Louisville School of Medicine, prepared the illustrations.

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The Serum Lipid Pattern in Hyperthyroidism, Hypothyroidism and Coronary Atherosclerosis*

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THE influence of thyroid activity upon the serum lipids has been well documented[1–5] and its clinical usefulness defined.[4–7] With the recent advent of new methods for describing the serum lipids with the analytic ultracentrifuge[8] and filter paper electrophoresis,[9] efforts are again being made to recognize some abnormality in the serum lipid pattern which might distinguish the hyperlipemia of myxedema from the hyperlipemia which may accompany atherosclerosis of the coronary arteries.[10,11] This would seem to be of more than passing interest in view of the current controversy over the relationship of hypothyroidism to atherosclerosis.[12,13]

MATERIAL AND METHODS

Fasting serum was obtained from patients in the University of Chicago Clinics who manifested hypothyroidism, hyperthyroidism or coronary artery disease. Over a three-year period samples were obtained from twenty-five patients, of whom twenty-one were females, with unequivocal hyperthyroidism as yet uncontrolled by therapy. The criteria for hyperthyroidism were the usual clinical signs supported by a basal metabolic rate of more than +20 per cent and/or a serum protein bound iodine level above 8 μg. per cent. Conversion ratios and data on thyroid uptake of I^{131} were obtained through the kindness of Dr. Dwight Clark when necessary.[14] Of these patients, three had an associated diastolic hypertension, one had the anginal syndrome and two had malignant exophthalmos.

In the same period, sera were obtained from seventeen patients with untreated hypothyroidism, of whom ten were females. Thirteen of these patients could be said to have true myxedema by the criteria of Gildea, Man and Peters,[6] including response to therapy. The remaining four were atypical only in that they had an equivocal therapeutic response. Of this total group, two were in congestive heart failure, one was troubled with the anginal syndrome, one patient had hypertension and two more had significant cardiomegaly. Thus approximately one-third of these patients had suggestive evidence of heart disease, although none had an electrocardiographic pattern diagnostic of previous myocardial infarction.

Many patients with coronary artery disease have been followed by periodic analyses of their serum lipids. From this large group were selected a few patients to be matched with the hypothyroid patients as to sex, age and serum cholesterol level. These groups were matched with some difficulty because of the sex difference in the two disorders and a difference in age distribution in the two groups. In order to match the high serum cholesterol levels found in certain hypothyroid patients, we included one man with a previous myocardial infarction and xanthoma tendinosum and tuberosum, and two women who had as yet manifested no evidence of coronary disease but had xanthoma tendinosum in one case and xanthoma planum in the other. One myxedematous woman, age thirty-three, could not be matched with any coronary disease patient, but was matched with a twenty-five-year old woman with comparable cholesterol level and a presumptive family history of coronary disease. The remainder of

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this matched group all had uncomplicated coronary artery disease (as defined by the New York Heart Association Committee of Diagnostic Criteria[15]), either proved myocardial infarction or the anginal syndrome. Sex, serum cholesterol level and age were matched as closely as possible although, as will be seen, certain cases were matched rather loosely as to cholesterol level or age. Except for three cases, which almost cancelled out, the serum cholesterol levels matched within ±31 mg. per cent. The age agreed within six years, except for four pairs. (None of several hundred cases of arteriosclerotic heart disease could be found to match closely with the seventy year old myxedematous man who had a serum cholesterol of 614. Consequently, he was compared in Table II with the younger man who had an essential hyperlipemia with xanthoma tuberosum and previous myocardial infarction.

Duplicate samples of serum obtained before treatment were extracted in alcohol:ether as described by Bloor[16] and analyzed in duplicate for total lipids, total cholesterol and lipid phosphorus. Total lipids were determined gravimetrically, cholesterol colorimetrically by the Lieberman-Burchard reaction, and lipid phosphorus colorimetrically using a modification of the Fiske-Subbarow technic. A third 5 cc. sample was then brought to a density of 1.21 with NaCl and KBr as described by Lewis and Page[17] and centrifuged for thirteen hours at 30,000 r.p.m. in the 30.2 rotor of the Spinco Model L centrifuge. The top fraction was removed and diluted to 2 cc. with salt solution of 1.21 density and analyzed in the Spinco Model E ultracentrifuge, exposures being made at 0, 2, 10, 26 and 34 minutes after reaching a running speed of 52,640 r.p.m. In determining concentration, no effort was made to correct for the Ogston-Johnston effect.[18]

TABLE I

COMPARISON OF THE MEAN OF THE SERUM LIPID FRACTIONS AND ITS STANDARD ERROR IN HYPOTHYROID AND HYPERTHYROID PATIENTS

	Number of Patients	Serum Lipids (Chemical Analyses) (mg. per cent)					Serum Lipoproteins (Ultracentrifugal Analyses) (mg. per cent)			
		Total Cholesterol	Total Lipid	Phospholipid	Per cent Phospholipid	C/LP	$-S_{1.21}$ 0–10 (α-Lipoprotein)	$-S_{1.21}$ 20–40 (β-Lipoprotein)	$-S_{1.21}$ 40–70	$-S_{1.21}$ 100–400
Hyperthyroidism: Mean	25	176	627	194	31.45	22.68	142	137	47	31
± Standard error	..	7.7	26	6.24	0.84	0.58	10.2	12	4.33	5.38
Hypothyroidism: Mean	17	408	1404	370	27.88	27.25	174.5	350	143	83
± Standard error	..	25.4	117	20.9	1.04	0.99	15.3	32	23.2	22.3

RESULTS

Since it is well known that serum lipid values may be normal in hyperthyroidism and occasionally in hypothyroidism, it seemed advisable to compare the series of hyperthyroid sera with the hyperthyroid samples, as had been done by Mason, Hunt and Hurxthal,[1] in an effort to find the serum lipid fraction most influenced by thyroid activity. Table I presents the mean values of the chemical and ultracentrifugal fractions in these two groups. It can be seen that there is a highly significant difference ($P < 0.01$) between all of the various chemical fractions, the beta lipoprotein, and its faster-rising components, $-S_{1.21}$ 40–70 and less certainly ($P < 0.04$) 100–400. There is no significant difference ($P > 0.1$) in the alpha ($-S_{1.21}$ 0–10) lipoprotein. The chemical fractions present a twofold increase in the hypothyroid group, the alpha lipoprotein a 1.25-fold difference, the

faster rising beta lipoprotein fractions approximately a threefold rise.

The difference in alpha$_1$ lipoprotein concentration is not significant but the hyperthyroid group has a greater predominance of women, and particularly of younger women who might be expected to show a higher alpha$_1$ lipoprotein concentration. Hence, the lesser difference between the two groups in this fraction might be explained in part by these differences in age and sex. It has been well demonstrated that estrogen administration will increase the concentration of alpha$_1$ lipoprotein in the serum of either sex,[19,20] and that the alpha$_1$ lipoprotein concentration is lower in young men than in young women.[17]

The greatest difference appears to be in the concentrations of beta lipoprotein ($-S_{1.21}$ 20–40), and its faster-rising component, $-S_{1.21}$ 40–70 (equivalent to Gofman's S_f 12–20), which are much higher in hypothyroidism. It has been suggested that these lipoproteins correlate with atherosclerosis.[13] It is worthy of note, however, that ten patients with hyperthyroidism presented $-S_{1.21}$ 40–70 levels greater than 50 mg. per cent and seven had an $-S_{1.21}$ 100–400 greater than 40 mg. per cent. These patients included three with malignant exophthalmos, two with "essential" hypertension and one with the anginal syndrome and electrocardiographic evidence of arteriosclerotic heart disease. Thus it would appear that an overactive thyroid is not incompatible with an elevation of the faster rising material with flotation rates from $-S_{1.21}$ 40–100.

Mention has been made of a large concentration of alpha$_2$ lipoprotein in the serum of hypothyroid dogs[11] and, in an isolated instance, in one myxedema patient's serum.[21] In general there was no over-all or consistent increase of alpha$_2$ lipoprotein in these hypothyroid sera—although it was present in measurable concentration (>10 mg. per cent) in about one-third of the cases, a frequency comparable to the matched group to be presented. (Fig. 1B.) However one patient previously untreated and undiagnosed, who was severely myxedematous, presented a large alpha$_2$ peak distinct from the beta peak. (Fig. 1D.) The alpha$_2$ peak almost equalled the beta peak in amplitude and the pattern was obviously unique. The chemical findings in this case were also unique, there being a large neutral fat component; the cholesterol was unusually low, relative to the total lipid. The alpha$_1$ concentration was quite low. This pattern of a large alpha$_2$ lipoprotein and a low alpha$_1$ is seen in some cases of "essential" hyperlipemia, in certain nephropathies and in certain cases of biliary cirrhosis. The low C/P ratio and high neutral fat suggests the latter most strongly in this patient. In this regard our experience corresponds with that of Swahn.[9] One patient with hyperthyroidism and mild essential hypertension also had a large beta peak which migrated slowly enough to perhaps include an alpha$_2$ peak. (Fig. 1A.)

Comparison between the two matched groups in Table II reveals a tendency to higher serum lipid values in the hypothyroid subjects than in the patients with arteriosclerotic heart disease. None of the differences noted are statistically significant except for the beta lipoprotein ($-S_{1.21}$ 20–40). The differences in cholesterol-phospholipid ratio and $-S_{1.21}$ 40–70 are significant only at the $P < 0.10$ level of confidence, the former being higher in atherosclerotic patients, the latter in myxedema. By Fisher's t test, it could be shown that the beta lipoprotein level was significantly higher ($P < 0.05$) in the arteriosclerotic group than in myxedema, although the tendency in the other fractions was for the myxedema group to be higher. The probability that the difference might be due to chance became even less ($P < 0.01$) when the beta lipoprotein/total lipid or beta lipoprotein/alpha lipoprotein ratios were used.

COMMENTS

One myxedematous subject's serum, of seventeen such patients, did show a large amount of alpha$_2$ ($-S_{1.21}$ 20–25) lipoprotein previously described in hypothyroid dogs[11] and humans.[21] Except for this unique case, none of the other patients showed any significant increase in concentration of this lipoprotein fraction. We have confirmed the observation that hypothyroid dogs present a large alpha$_2$ fraction but, in fact, even normal dogs do not carry lipids with a beta globulin, but with the alpha globulins; hence the alpha$_2$ lipoprotein grows larger in induced canine hyperlipemia, e.g., diabetes. Also there is a wide variation in the flotation rate of the major (beta lipoprotein) peak from human to human, and sometimes in the same person. In some human sera we have seen an apparent reduction in rate of movement of the peak's nadir with increasing concentrations of the major component (Fig. 1C) and it becomes difficult

to separate this from the case in which the alpha$_2$ peak may be combined with the beta peak, defying resolution. (Fig. 1A.) This situation must be distinguished from an unquestioned second peak in the $-S_{1.21}$ range of 20–25 existing in the presence of a well defined beta lipoprotein peak at $-S_{1.21}$ 25 to 38. (Fig. 1D.) We have seen this consistently in three cases of hepatobiliary disease we have studied and in six patients with essential hyperlipemia, four of whom had suffered with symptoms (and had electrocardiographic evidence) of coronary artery disease. One of these patients apparently had a healed pyelonephritis.

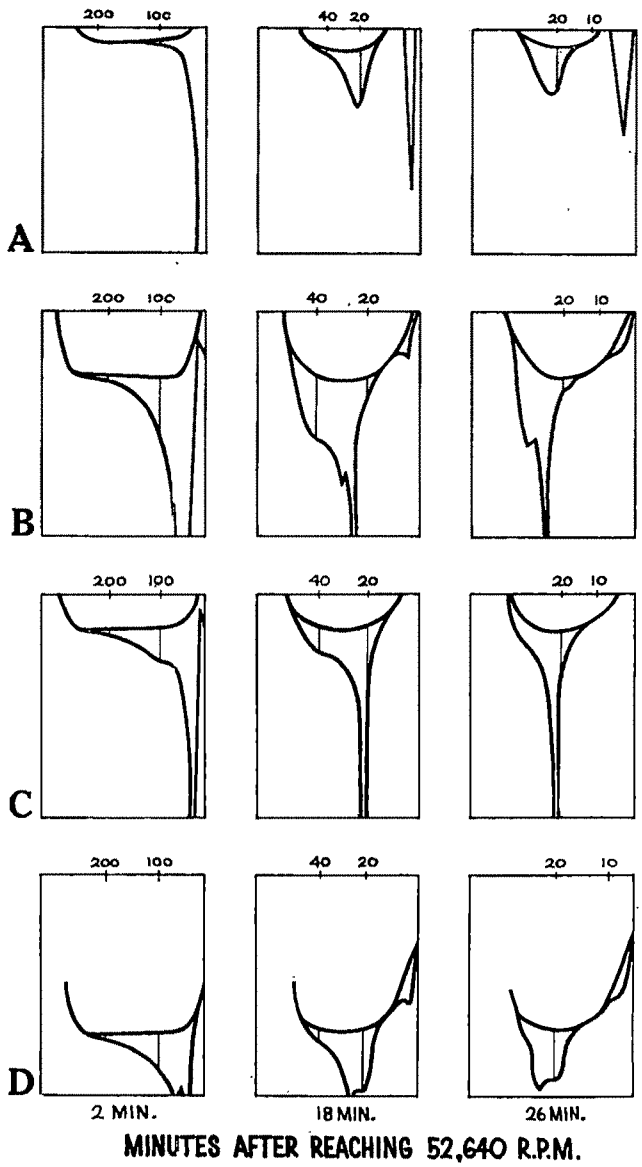


FIG. 1. Serum lipoprotein patterns in four patients. Flotation rate at density 1.21 and 26°C. A, fifty-six year old woman with essential hypertension and hyperthyroidism. (B.M.R. +71.) B, sixty-five year old female with myxedema and arteriosclerotic heart disease. (B.M.R. −30.) C, sixty-three year old female with myxedema. (B.M.R. −38.) D, fifty-seven year old man with myxedema, spondylolisthesis, right hip pain and inguinal hernias. (B.M.R. −36.)

TABLE II

COMPARISON OF HYPERLIPEMIC PATTERNS IN HYPOTHYROID PATIENTS WITH PATTERNS IN FAMILIAL HYPERCHOLESTEREMIA PATIENTS WITH CORONARY ARTERY DISEASE*

Patient		Sex and Age	Cholesterol (mg. %)	Total Lipid (mg. %)	Phospholipid (mg. %)	α Lipoprotein $-S_{1.21} < 1$ (mg. %)	β Lipoprotein $-S_{1.21}$ 20–40 (mg. %)	$-S_{1.21}$ 40–70 (mg. %)	$-S_{1.21}$ 100–400 (mg. %)	β Lipoprotein / α Lipoprotein Ratio	Per cent β Lipoprotein
Hypothyroidism	Coronary Disease										
Keu		F, 31	389	1098	328	246	230	209	322+	0.94	21.0
	Gre†	F, 25	337	963	320	295	365	63	30	1.24	38.0
Fai		F, 33	309	988	290	208	347	79	15	1.67	35.1
	Dav	F, 38	288	1020	303	208	598	67	110	2.87	58.6
Kam		F, 35	413	1485	360	155	475	213	55	3.07	32.0
	Lic‡	F, 27	480	1245	323	60	518	113	3	8.63	41.6
Sta		F, 44	654	2243	596	162	577	204	58+	3.56	25.7
	Keu	F, 60	646	2185	505	81	672	213	310	8.30	30.7
Gri		M, 48	540	1537	382	107	416	116	88+	3.89	27.1
	Cre	M, 25	571	1328	390	154	644	40	0	4.18	48.5
Hor		F, 50	412	1143	328	244	307	67	43+	1.26	26.8
	Hee	F, 52	393	1043	325	154	539	67	24	3.50	51.1
Tob		F, 50	362	1103	310	205	436	85	40	2.13	39.5
	Che	F, 50	371	987	301	132	307	38	0	2.32	31.2
Lew		M, 52	371	1090	302	188	374	106	3	1.99	34.3
	Ros	M, 51	355	1043	298	158	353	73	6	2.23	33.8
Fal		F, 56	421	1158	378	252	205	60	12	0.81	17.7
	Bak‡	F, 51	438	1328	365	161	769	146	18	4.77	58.0
Hal		M, 57	327	2520	472	93	180	234	255	1.94	07.2
	Ric	M, 53	340	1213	310	121	322	79	30+	2.66	26.5
Tob		M, 58	300	841	305	143	196	36	10	1.37	23.3
	Par	M, 55	313	1130	285	57	432	140	209	7.58	38.2
Cel		F, 60	400	1185	360	290	176	75	26	0.61	14.9
	Lou	F, 63	372	1140	367	380	599	128	9	1.58	52.5
Rig		M, 62	319	1290	298	67	390	140	52	5.32	30.2
	Ada	M, 60	311	913	255	107	295	103	48	2.76	32.3
Con		F, 63	410	1425	365	222	417	137	118	1.38	29.3
	Bab	F, 60	410	1058	338	228	392	104	16+	1.72	37.1
Kuc		F, 65	408	1875	480	108	611	394	106	5.66	32.6
	Car	F, 54	381	1050	308	100	410	115	52	4.10	39.0
Qua		M, 69	285	905	275	151	356	30	15	2.21	37.2
	Pax	M, 65	297	1008	300	165	304	55	24	1.81	30.1
Blu		M, 70	614	1990	463	129	289	249	193	2.24	14.5
	Bar	M, 44	534	2623	525	62	377	258	1010+	6.07	14.3
Mean:											
Hypothyroidism		53	408	1404	370	174.7	350.7	143	83	2.415	26.3
Coronary disease		49	402	1252	342	154.5	464.5	106	112	3.901	38.9
± Standard error:											
Hypothyroidism		..	25.8	118	20.9	15.5	32.0	23.2	22.3	0.373	2.2
Coronary disease		..	24.6	111	17.8	20.8	36.0	14.4	59.6	0.581	2.8

* Patients matched as to sex, age and serum cholesterol level.
† Family history only of coronary disease.
‡ Xanthoma tuberosum without proved coronary disease.

The one patient with myxedema who presented a definite double peak of the major lipoprotein present, the slower peak of the two falling in the centrifugal range of alpha$_2$ lipoprotein (Fig. 1D), had none of the aforementioned complications clinically but did suffer from arthritis of obscure etiology which involved the back and right hip. One of the hyperthyroid patients who had essential hypertension presented a large slow moving peak (Fig. 1A), the nadir of which occurred at $-S_{1.21}$ 23 and could be interpreted as a combination of alpha$_2$ and beta lipoproteins which could not be resolved. The alpha$_1$ lipoprotein is apparently little affected by thyroid activity although our data are not designed to test this conclusively.

In all situations in which hyperlipemia or hypercholesteremia exist, the tendency is for other fractions, both chemical and ultracentrifugal, to rise concurrently. This tendency can be seen in either coronary atherosclerosis or myxedema, yet the ultracentrifugal studies offer finer differentiation. While it may be said that the hypercholesteremia of both groups is associated with an increase in serum beta lipoprotein, and we would agree with Malmros and Swahn[10] that the lipid pattern is similar in hypothyroidism and atherosclerosis, yet it may be further noted that the beta lipoprotein level is proportionately much higher in atherosclerosis for a given degree of hypercholesteremia. This lends further support to the observations of Pratt,[22] Eder, Russ and Barr[23] and the conception of Page[24] who suggested that the primary abnormality of the serum lipids in coronary atherosclerosis may be an excessive beta lipoproteinemia, either relative to the level of alpha lipoprotein or absolutely. Gofman's fraction, our $-S_{1.21}$ 40–70, seems to mirror the beta lipoprotein in general and should probably be considered a portion of that moiety. This, coupled with the fact that certain hyperthyroid patients still manage to have abnormal levels of $-S_{1.21}$ 40–400, suggests that the mechanism of development of the serum lipid abnormality differs in atherosclerosis from the hypercholesterolemia of myxedema.[25] However, if the "intimal infiltration" theory of atherogenesis is correct, and a particular lipid fraction of the serum is more culpable than others in atherosclerosis, any lipoprotein or chemical lipid fraction found associated with atherosclerosis can also be found, and in high concentrations, in myxedema. In other words this difference is only relative, not absolute, and in no way helps to settle the controversy as to the atherogenicity of the hypothyroid state.

SUMMARY

Serum lipids and serum lipoproteins were studied in twenty-five hyperthyroid and seventeen myxedema patients. Mean total lipid, total cholesterol and phospholipid levels were all significantly higher in the latter group. The beta lipoprotein and faster rising lipoprotein moieties showed a particularly striking increment, and contrasted with an insignificant difference in the alpha lipoproteins.

The increase of the serum lipids in patients with hypothyroidism is qualitatively indistinguishable from that seen in idiopathic hypercholesterolemia which is so often associated with coronary atherosclerosis. However, when such hypercholesteremic patients are matched with hypothyroid patients as to sex, age and cholesterol level, a significantly higher beta lipoprotein fraction is found in the former group. This suggests that the hyperlipemia of atherosclerosis arises through a mechanism which is different from that of myxedema. This observation is also consistent with the idea that the primary abnormality of the serum lipids in atherosclerosis is an elevation in the level of beta lipoprotein.

Acknowledgment: We are grateful for the excellent technical assistance of Sheldon Huffman, Eugene Balter, Dr. Lois Sheimann and Irene Carr in certain phases of this work.

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The Night-eating Syndrome*

A Pattern of Food Intake among Certain Obese Patients

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NONE of the numerous recent studies of obesity has dealt with the eating patterns of obese patients in more than a cursory manner. The rare papers on the subject have reported that obese patients eat more frequently than the non-obese,[1] less frequently,[2] or even that decreased activity rather than a change in eating habits leads to obesity.[3] In two papers incidental mention has been made that obese patients eat more at night.[4,5]

Observation of twenty-five obese patients at a special study clinic of the New York Hospital has disclosed that the eating patterns of a large number form a distinctive syndrome characterized by nocturnal hyperphagia, insomnia and morning anorexia. Such a pattern was not found in any of thirty-eight subjects without history of weight disorder. This "night-eating syndrome" was particularly prominent during periods of weight gain and life stress. An effective reducing regimen was not possible in its presence, and it appeared to be intimately related to the pathogenesis of obesity.

METHOD

The subjects of this report were seen in a special study clinic of the New York Hospital where they had been sent from other departments because of the severity of their obesity or difficulty in its management. The group thus includes a high proportion of emotionally disturbed individuals, but whether this proportion is higher than in any group of comparably severe obese patients is conjectural.

The report includes all obese patients referred to the clinic who were seen at least four times. It should be noted that only two patients failed to keep at least four appointments, so that the series comprises 93 per cent of the patients initially referred, including the only two men. The reason for the great preponderance of women is not clear.

The median age of the patients was thirty-five years (range eighteen to fifty-six). Percentage of overweight was determined from the tables of the Medico-Actuarial Mortality Investigation of 1912[6] which used clothed subjects in shoes. Following Keys,[7] the current study used these standards for lightly clothed, barefoot individuals without correction for either height of shoes or weight of clothes. The resulting average value of 68 per cent overweight (range 22 to 137) is thus lower than would be calculated from standards of "ideal" weight.

The study of each patient included a detailed history and physical examination, together with routine laboratory tests and any indicated special procedures. Most of the patients were seen for an hour every week or two over a period of six months. Three had only four interviews, and two (H. G. and M. B.) had more than 100. Medication was prescribed when indicated but the focus of the treatment was supportive psychotherapy combined with environmental manipulation. In a few of the patients more intensive psychotherapy was attempted. (Table I.)

Data about the patient's diets were obtained from three sources: the patient's record of food intake, the routine dietary history in the nutrition clinic, and a careful study of eating habits during the treatment period. The intake was often recorded as much as 400 calories per day higher by the second method, and 800 calories higher by the third. Such an increase in the caloric intake reported by obese patients when subjected to more detailed inquiry agrees well with published reports[8] and attests to the accuracy of the information obtained during the treatment periods. Reducing diets were prescribed only on the patient's request and the

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physician attempted to place the patient under no obligation to adhere to the diet. These were usually standard diets of 1,200 calories, containing 70 gm. of protein, 45 gm. of fat and 130 gm. of carbohydrate.

amounts of food during the evening and night and no one was considered to manifest the syndrome unless he ate at least a quarter of his total calories for the day during the period following the evening meal. Thus a significant part

TABLE 1

No.	Patient*	Age	Height	Weight (lb.)	Over-weight (%)	Components of Syndrome Present	Weight Loss in Clinic	Previous Maximal Weight Loss	Complications
1	D. M.	23	5′ 0″	275	137	2 (sometimes alcohol instead of food)	0	70 lb.†	0
2	H. G.	44	4′ 10½″	290	130	3	0	80, 150 lb. (in hospital)†	See case report
3	L. M.	30	5′2″	260	110	2 (no insomnia)	0	50 lb.	"Neurasthenia"
4	M. B.	18	5′ 0″	230	105	3	30 lb.	0	0
5	E. S.	30	5′ 6½″	280	100	3	30 lb. (in hospital)	30 lb.	0
6	M. L.	25	5′ 5″	255	95	3	0	60 lb.†	"Neurasthenia"
7	S. B.	36	5′ 7½″	283	90	0	45 lb.†	50, 45 lb.†	0
8	F. W.	48	5′ 5½″	290	86	0	45 lb.†	80 lb.†	0
9	E. M.	36	5 2½″	238	84	3	0	50 lb.†	Psychotic depression
10	A. R.	28	5′ 4″	235	81	2 (no insomnia)	10 lb.	50 lb.†	Severe anxiety
11	E. H.	35	5′ 3″	235	81	0	0	0	0
12	L. S.	27	5′ 2½″	220	77	3	12 lb.	18 lb.	"Neurasthenia" previously, asthma and bleeding ulcer in clinic
13	A. F.	56	5′ 3½″	245	71	0	105 lb.†	0	0
14	S. V.	31	5′ ½″	198	62	3	0	0	0
15	M. A.	42	5′ 1″	200	54	3	0	0	0
16	O. A.	38	5′ 11″	262	51	3	20 lb.	45 lb.†	0
17	S. M.	51	5′ 2½″	204	46	3	0	0	0
18	L. C.	30	4′ 9½″	166	43	0	50 lb.†	75, 45 lb.†	0
19	M. C.	49	5′ 0″	183	39	3	40 lb.†	0	Psychotic depression
20	S. G.	34	5′ 3″	176	35	3	0	70, 45 lb.†	Depression (2×)
21	I. G.	50	5 1″	180	33	3	0	0	0
22	M. P.	26	5′ 5″	175	32	3	20 lb.†	0	0
23	R. G.	18	5′ 2″	148	26	3	0	0	0
24	S. S.	43	5′ 2½″	166	22	2 (sometimes alcohol instead of food)	0	28 lb.†	"Neurasthenia"
25	B. S	54	5′ 5″	180	22	3	0	14 lb.	0

* All patients were women with the exception of No. 8 and No. 16.
† At least one-third of excess weight.

RESULTS

Definition of Syndrome. An effort was made to establish gross criteria for the three major findings which comprise the night-eating syndrome and which might serve to distinguish it from other patterns of eating. First, and of greatest importance, is the consumption of large of the total caloric intake, and a major part of the excess intake, occurs during a time when the food intake of non-obese people is negligible. The second criterion is sleeplessness, at least until midnight more than half of the time, and the third is morning anorexia with negligible food intake at breakfast. In no case did this

include more than a cup of coffee and a small glass of orange juice.

Specificity of Syndrome. The eating pattern of the obese group was compared with that of thirty-eight subjects who had no past history of weight disorder. This latter group was composed of graduate and student nurses routinely examined in the personnel health clinic of the medical center. All of the subjects were women and the median of their ages was twenty-four years, ranging from twenty-one to forty. The majority were residents in the nurses' home where they were able to obtain meals when they were off duty. In addition, permission to keep food in their rooms and a refrigerator on each floor allowed food intake to be largely independent of the regular cafeteria hours. Further evidence that the findings in the control group were not due to inaccessibility of food is provided by the rather high incidence of obesity in the nurses' home.

Of the thirty-eight control subjects, not one reported the night-eating syndrome. In considering the components of the syndrome, most of the group gave a history of food intake following the evening meal, commonly a sandwich and glass of milk before going to bed, but in no case did this approach 25 per cent of the daily food intake. One of the group reported insomnia. Nine reported that they had little or no appetite for breakfast but three of these said that they ate a substantial breakfast anyway.

Incidence of Syndrome. The night-eating syndrome in its entirety was reported by sixteen of twenty-five patients, and it was present with minor variations in another four patients. Of these latter four, two patients often consumed alcohol instead of food during the evening while two others did not report insomnia despite a large caloric intake following the evening meal.

Although these twenty patients reported that the night-eating syndrome occurred much of the time, it was not invariably present. Accordingly, an attempt was made to determine in what circumstances it was most likely to occur. Most of the patients agreed that they ate at night during two closely related situations—periods of weight gain and periods of life stress.

All twenty of the patients who reported night eating said that it was the major pattern during periods of weight gain. Two of the remaining five patients (S. B. and L. C.) said that during their periods of weight gain they ate increased amounts at mealtimes, one (F. W.) drank large amounts of alcohol without restricting food intake, one (A. F.) ate indiscriminately throughout the day and one (E. H.) maintained her usual food intake while at bedrest for treatment of pulmonary tuberculosis. Eighteen of the twenty patients exhibiting the night-eating syndrome said that it was most conspicuous during periods of life stress. Both of the patients (B. S. and L. M.) who did not make this correlation were denying obvious emotional disturbances while acknowledging presence of the syndrome.

Night-eating Syndrome and Prognosis. Presence of the night-eating syndrome influenced (1) results of weight reduction programs and (2) the incidence of untoward reactions to such programs.

1. Results of weight reduction programs: Loss of one-third of patient's excess weight was selected as indicating modest success of a weight reduction program. By these standards only six of the twenty-five patients reduced successfully. Such results are poorer than those in other series,[9–13] a circumstance perhaps arising from the selection of a large number of patients who had already proved difficult to manage. The high incidence of the night-eating syndrome in this group may reflect this same selection, for there is a striking correlation between presence of the syndrome and failure of dietary regimens. Thus of the twenty patients reporting the syndrome, only two were able to lose more than one-third of their initial excess weight. In one of these (M. C.) the weight loss occurred in the course of a psychotic depressive reaction; in the other (M. P.) it totaled only twenty pounds in a woman who was initially one of the least overweight. In contrast, of the five patients who did not report the pattern, four (M. B., E. S., A. R. and O. A.) lost an average of sixty-one pounds, or 67 per cent of their excess weight.

Five patients manifesting the night-eating syndrome lost from ten to thirty pounds without being able to maintain a reducing regimen. Their experience throws light on the significance of night eating as an index of refractoriness to weight reduction programs. Thus it was during temporary remissions in the syndrome that this weight loss occurred in four of these five patients.

The patients' past histories of attempts at weight reduction were similar to the findings during treatment in the clinic. Of the twenty patients exhibiting the syndrome, only eleven

had ever succeeded in losing more than one-third of their excess weight. On the other hand of the five patients not reporting the syndrome four had carried out successful weight reduction programs in the past.

2. Untoward reactions to weight reduction programs. Untoward reactions to weight reduction in obese patients have recently been given prominence, largely through the publications of Bruch.[14,15] The prevalence of such responses is emphasized by the experience of the patients in the present series, who suffered a total of ten severe complications during previous and current efforts at weight reduction.

Considering first the results of treatment of night-eating patients in this clinic, one (M. C.), as already noted, suffered a psychotic depressive reaction two months after she began to diet. Another (L. S.), who had lost 12 pounds in the first month of a reducing regimen, was forced to stop dieting by the onset of severe bronchial asthma and bleeding peptic ulcer. There were no untoward reactions among the five patients not showing the syndrome.

Dramatic evidence of the influence of the night-eating syndrome on the incidence of untoward reactions to dieting is obtained from the history of the patients' past experience with reducing regimens. As has been noted, only eleven of twenty patients with the night-eating syndrome had previously been able to lose more than one-third of their excess weight. In eight of these eleven instances this weight loss was accompanied by disabling emotional illness. Four of the patients presented a remarkably similar picture of neurasthenic type characterized by feelings of futility, depression of mood and inordinate fatigue leading to confinement in bed for as long as four months. Three patients stopped weight-reducing regimens because of depressive reactions, and one following the onset of severe anxiety. Therefore, of eleven patients with a past history of significant weight loss, only three achieved this without disabling complications. One of these three patients (H. G.), reported subsequently, experienced severe anxiety in the course of a reducing regimen but factors other than diet seemed primarily responsible.

In contrast to this startling incidence of disability during attempts at weight reduction among patients with the night-eating syndrome is the infrequency of such complications in the group not showing the pattern. Although this latter group contained only five patients, four had previously lost significant amounts of weight without complication.

CASE REPORTS

The following two case reports illustrate various aspects of the night-eating syndrome. In the first, prolonged dietary restriction leading to a marked weight loss did not abolish the syndrome or prevent its intensification in a situation of life stress.

CASE 1. H. G. was a forty-three year old single woman clerical worker who was observed over a two-year period in another hospital to which she had been admitted for treatment of cardiac failure on the basis of hypertensive disease and massive obesity.

The patient was the eldest of four children born to an orthodox Jewish family in a small midwestern city. Her father, a quiet, bookish man took little part in the family life; her mother, on the other hand, "ran everything."

Childhood and adolescence were characterized by vacillating and inconsistent parental care, and intense, unsuccessful rivalry with a younger brother. At the age of twenty-one the patient suffered severe lacerations in an automobile accident, to which her mother reacted with great distress. Soon thereafter the mother developed incapacitating cardiac symptoms which she blamed on worry over the patient, and for the next nine years she remained a semi-invalid. During this time she induced the patient to devote herself wholly to her care and to abandon her hopes for independence, a career and marriage. Some months after her mother's death the patient, then thirty-one, learned that the doctor had considered her mother's long-standing condition a neurosis, and her symptoms largely exhibitionistic and exploitative. The patient felt betrayed and bewildered, and left home to come to New York.

During the ensuing seven years she gained weight from 200 to 400 pounds (height 4 feet 10½ inches) by overeating in a pronounced night-eating pattern. Thus she rarely ate before noon, and at supper began her large intake of sweet foods which continued until she went to bed late at night. During these hours she was assailed by loneliness and anxiety. To lessen the distress she kept someone with her as much as possible and, when she was alone, left the door and windows open, all the lights burning and played the radio loudly. She rarely fell asleep

before midnight and usually woke within an hour, anxious and hungry. At such times she would eat a pint of ice cream, drink a bottle of Pepsi Cola and, temporarily sated, fall asleep for another hour before the cycle was repeated. She awoke three or four times a night in this manner.

By the age of thirty-five the patient's obesity and hypertension had so disabled her that she sought medical attention, and for a period of six years she received general medical and psychiatric care as an outpatient. Ultimately cardiac failure became so severe that hospitalization was necessary. This only partially decreased the nocturnal anxiety and hunger but rigid caloric restriction succeeded in effecting a loss of 150 pounds over a period of one year. At this time two traumatic events occurred: a physician to whom the patient had become much attached left the hospital, and the patient underwent an operation for umbilical hernia which was complicated by wound infection. She reacted with intense anxiety and petulant, infantile behavior. These were most marked at night when she would spend as much as four hours tearfully pacing the corridors with intermittent futile attempts at reading and knitting. As a therapeutic trial, free access to food was permitted and the patient immediately returned to the night-eating pattern. This was associated with a weight gain of 50 pounds in the course of the next two months and no apparent improvement in her emotional disorder.

In the following patient remission and exacerbation of the night-eating syndrome followed promptly upon changes in her life situation, and were associated with marked variation in the success of a dietary regimen.

CASE II. E. S. was a twenty-nine year old housewife of Irish ancestry who was treated in a special study clinic of the New York Hospital over a period of one year. She came for treatment of her obesity as a result of urgent medical advice following recurrent severe attacks of thrombophlebitis.

The patient was the eighth of ten living children of a mother who had had ten additional pregnancies. Her restrictive and arbitrary father worked as a truck driver but the income from his sporadic employment never permitted the family more than a marginal existence. The patient remembered her childhood as a time of hardship and deprivation, and of great bitterness toward a mother who, she felt, exploited her. At the age of eighteen she married the neighborhood alcoholic in an effort to escape this home environment and soon found herself in an even more unpleasant situation. During the next eight years she gained 100 pounds, attaining a weight of 280 pounds (height 5 feet, 6 inches), which she maintained during the three years before admission to the clinic.

The patient's manner was pleasant and friendly during the treatment periods but she did not participate actively, and her communications had a curiously impersonal quality. She was usually late to appointments and attendance was irregular.

Her pattern of food intake was characteristic of the night-eating syndrome. During periods of emotional turmoil, in which the patient was either gaining weight or trying unsuccessfully to lose it, she awoke in the morning completely anorexic and ate no breakfast and little lunch. Supper, which she ate in the early evening, was large, yet provided only transient satiety. During the rest of the evening, and often until early morning, she nibbled at various foods distinguished mainly by their sweetness. The patient was unclear about the specific incentive to eating at these times but suggested that it was due to the anxiety she felt at night. This was most severe when she was alone, and was often experienced as a fear of someone breaking into the house to harm her. None of the measures she took provided more than partial relief; these included, besides eating, having company in the house and, when she had to be alone, keeping the radio and lights on. The presence of her husband sometimes reassured her but often quarrels with him had precipitated her anxiety and at such times he served only to upset her further. Insomnia and eating continued until at least midnight, often until two or three in the morning, and throughout the treatment period she rarely slept more than four hours.

After the patient had been receiving treatment for seven months a recurrence of phlebitis developed which made it necessary for her to enter the hospital. She greeted this development with relief and gladly laid down her domestic responsibilities. Within a day there was a striking change in the eating pattern. The morning after admission the patient awoke with a desire to eat breakfast for the first time in months, went on to consume a small lunch and supper, and had no desire to eat during the

evening or night. This was more remarkable in that the pain in her legs kept her awake much of the night during the first week of hospitalization. Thereafter she slept well and at no time complained of hunger. She adhered without difficulty to an 800 calorie diet and lost twenty-eight pounds in thirty-one days.

This marked change in eating pattern was paralleled by a great decrease in free anxiety and, despite her pain, the patient seemed more at ease than at any time during the previous months as an outpatient. She attributed this change in both comfort and eating pattern partly to the experience of hospitalization and partly to the presence of a roommate. "Mrs. C. keeps me feeling good in the evenings by talking to me so I don't feel like eating."

The patient made every effort to remain in the hospital; and when she finally returned home, it was with greatest reluctance. Within twenty-four hours the night-eating syndrome recurred The morning after her arrival home she awoke anorexic, did not begin to eat until evening and stayed up until 3:00 A.M. nibbling. Her husband, who had stopped drinking while she was in the hospital, began again soon after her return, and relations between them which had improved during her hospitalization deteriorated rapidly. During the first month following her discharge the patient gained weight at a rate of 2 pounds a week.

COMMENTS

In recent years there has been a growing appreciation of the emotional disturbance in many overweight people and even of the fact that their excess weight has been gained in relatively short periods of positive caloric balance. In point of fact, a large number of obese patients are losing weight much of the time and it is short periods of rapid weight gain which undo the results of weeks and months of dieting. If obesity be considered a scar of preceding periods of positive energy balance, it is just these periods which are of significance in its pathogenesis. Yet the conditions existing during these crucial times are largely obscure, as can be judged from the fact that the night-eating syndrome described here has not been reported previously. This is the more remarkable in that it is well known to the obese patients themselves, as well as to those relatives, nurses and dietitians who have much contact with them.

The major significance of the night-eating syndrome may lie in its relation to the life situation of the individual who manifests it. Although it is not present all of the time, even in the very obese, occurrence of the syndrome can be predicted during periods of weight gain and life stress, and these three variables are linked with remarkable consistency. What, then, is their interrelationship?

The relation between the eating pattern and life stress appears clear. They regularly occur together, and although the obese patients may respond to stress in other ways as well, when the night-eating syndrome is found, it is in situations of life stress.

The relation of these two factors to the periods of weight gain is less immediately apparent. Although it is difficult to conceive of life stress as being a consequence of weight gain, night eating could be. The experience of the two cases cited, however, argues against this. Thus the first patient (H. G.) manifested the night-eating syndrome at intervals over a year's time despite forcible restriction of food intake resulting in a weight loss of 150 pounds. In the second patient (E. S.) the syndrome disappeared within a day and reappeared as promptly following changes in her life situation, hardly long enough for changes in weight to have had an effect. It therefore seems justified to conclude that the night-eating syndrome is not a result of weight gain, overeating or any metabolic consequence of these. The syndrome appears rather to represent a response to stress of a type peculiar to certain obese patients, and one intimately related to the overeating which leads to their obesity.

Alterations in diurnal rhythms in response to stress are known to the clinician but they are not common. Two which have received some prominence are the sleep reversal following encephalitis and the "morning-evening variation" of the depressive reaction. The night-eating syndrome appears distinct from these two. None of the patients in this series had a history compatible with encephalitis. And while their food intake increases during the day, as that of depressed patients, their distress also increases, in contrast to the experience of patients with depression.

That the normal diurnal eating pattern may be important in the maintenance of caloric balance is suggested by an observation of Brooks.[16] This author has noted that destruction of the ventromedial nuclei of the hypothalamus

abolished the normal pattern of nocturnal eating in the rat. During the subsequent period of weight gain in the so-called hypothalamic obese rat the normal pattern is replaced by chaotic eating throughout the twenty-four hours.

Significance of Syndrome for Weight Reduction. It has long been recognized that there are many patients who cannot maintain a reducing diet long enough to make it worth either their effort or the doctor's time. However, it has only recently been pointed out by Bruch that in addition to these patients who cannot lose weight there is a group of emotionally disturbed obese patients who should not even try. There is too great a danger that such efforts, "if rigidly enforced . . . may precipitate serious mental disturbance."

There are unfortunately no criteria to select those patients who might undertake dieting with safety and reasonable hope of success. If the results of this study are confirmed in a series including a larger number of obese patients not manifesting the night-eating syndrome, recognition of the syndrome could be of value in deciding whether to restrict food intake. The presence of the syndrome in an obese person indicates that it will be very difficult for him to lose weight, and that he may attempt it only at a risk far outweighing the benefits to be derived. However, the fact that the syndrome is not always present, even in those persons who may exhibit it much of the time, suggests that dieting may not be permanently contraindicated for such individuals. On the contrary, the sensitivity with which it reflects the emotional state of the patient might allow the syndrome to serve as an index of the success of psychotherapeutic measures. Thus remissions could be taken as indication that weight reduction might reasonably be attempted. Finally, reducing diets can be prescribed with confidence for those patients who have never manifested the syndrome.

Dexedrine®. The most widely prescribed drug in the treatment of obesity is probably dexedrine, a circumstance dependent upon its well recognized action in depressing appetite. Reports of its value in actual practice have, however, varied widely.[5,17] Demonstration of a night-eating syndrome in many obese patients may help to reconcile these conflicting findings as well as provide a better rationale for use of the drug in weight reduction programs. If the patient does a major part of his eating at night, in conjunction with anxiety and insomnia, a drug which promotes sleeplessness is of doubtful value and its use during the period of morning anorexia is little more than a gesture. On the other hand, when the patient's overeating occurs during the daylight hours, the maximum appetite-depressing effect of dexedrine is obtained without the undesirable side effects.

Estimation of Caloric Intake. A common and well recognized characteristic of obese patients is their tendency to underestimate their caloric intake. Although this can be explained easily enough on the basis of the shame involved in acknowledging how much they have eaten, recognition of a night-eating syndrome may throw further light on the problem. Beaudoin[8] has shown that underestimation of caloric intake by obese subjects is greatest in regard to food eaten during the latter part of the day. She adduced no reason for this finding but Bruch's investigation of certain obese patients has revealed a marked repression of the knowledge of food eaten at night.[18] This is associated with early feeding problems, eating as defiance, and the later unconscious rationalization that "food eaten after dark does not count." Some such selective inattention renders more understandable reports of food intake in the 1,200 calorie range by patients who continue to gain weight. This is perhaps all they do eat during the daylight hours.

Relation to Research Procedures. Presence of the eating pattern described here has certain implications for research in obesity. Thus investigations made during the morning and early afternoon hours may give information about the patient which is quite different from that which would be found by investigation during the evening and night hours when he is overeating.

Tumor of the Pancreas. Since the concept of endogenous obesity has fallen into disrepute, the differential diagnosis of obesity has involved few considerations of structural disease. One notable exception has been the obesity associated with insulin-secreting tumor of the pancreas. In this condition hunger and maximum food intake classically follow periods of food deprivation, most notably during the morning hours either before breakfast, or in its absence. Recognition of the night-eating syndrome, with its morning anorexia, therefore, is helpful in excluding the possibility of organic hyperinsulinism.

Etiology. The etiology of the night-eating syndrome has not been determined. Furthermore, its characteristics do not reveal whether the

mechanisms involved in its pathogenesis are primarily physiologic or psychologic. Thus disordered carbohydrate metabolism involving increased tolerance to glucose has been reported in certain cases of obesity.[19,20] If, as seems likely, utilization of carbohydrate is under the control of hormones which are in part regulated by the intake of carbohydrate, there must be a mechanism which inactivates or decreases the secretion of these hormones with a failing carbohydrate supply. Such a sequence would involve the formal properties of a servomechanism as described by Wiener,[21] and in normal function would involve utilization of negative feedback to maintain the system in equilibrium. Disturbance in the feedback mechanism could lead to breakdown of the system in such a way that recurrent bouts of hypoglycemia would succeed each other in accelerating succession. Such changes might find expression in the periods of decreasing satiety noted during the evening. When sleep finally interrupted the intake of carbohydrate, the reactive hypoglycemia would cease, and the blood sugar would remain stable throughout the morning period characterized by anorexia.

Another explanation of the night-eating syndrome is suggested by the observation that the evening anxiety and impulse to eat often can be interrupted by the most simple interpersonal means. Talking with the physician at midnight may be as useful as massive sedation in temporarily alleviating both the anxiety and the need to eat. The period of going to bed, the evening, is traditionally associated in children with the greatest anxiety, presumably due to the fear of separation from the parent and the threat of the breakthrough of anxiety-productive conflict, as in dreams and nightmares. The obese women, in this group at least, were for the most part remarkably immature characters who relied inordinately on praise and tokens of love for the control of anxiety and the regulation of self-esteem. It seems possible that, as in children, the breakthrough of anxiety during the evening may result from this same interruption of their source of affection and approval. It is unlikely that the night-eating pattern is more directly related to a childhood one, as none of the patients could definitely remember such an eating pattern in childhood, nor had this period been characterized by a greater incidence of night terrors or bedtime conflict than would be expected in any comparable group.

SUMMARY AND CONCLUSIONS

1. A distinctive eating pattern of obese patients is described. This "night-eating syndrome" is characterized by nocturnal hyperphagia, insomnia and morning anorexia.

2. The syndrome was present in twenty of twenty-five obese patients treated in a special study clinic and in none of thirty-eight non-obese subjects.

3. Patients manifesting the syndrome had great difficulty losing weight and experienced a high incidence of complications in their attempts.

4. It is suggested that the syndrome represents a response to stress of a type peculiar to certain obese patients, and that it is intimately related to the pathogenesis of their obesity.

Addendum: A description of morning anorexia and evening hyperphagia in obese patients recently appeared in a paper entitled "Treatment of Obesity with a Low Protein, Calorically Unrestricted Diet," by V. P. Dole, I. L. Schwartz, J. H. Thaysen, N. A. Thorn and L. Silver, *Am. J. Clinical Nutrition*, 2: 381, 1953.

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Review

The Metal Chelate Compounds of Urine*

Their Relation to the Initiation and Growth of Calculi

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URINARY calculi are composed of an organic matrix and a crystalline body. This matrix may be an inert inclusion present by chance association with the growing stone, or may possibly serve in a purely physical role as an adhesive binder to hold the crystals in an organized structure.[13] That it may utilize chemical bonds to play a more active role in the formation and growth of calculi is suggested by the recent investigations of Howard[14] and Engel.[10,11]

No scientific approach to the prevention of urinary calculi seems possible until this question of the chemical activity or inertness of the organic matrix has been answered. It is the purpose of this report to correlate some of the biochemical and physicochemical properties of the matrix with those of the crystalline components of urinary calculi.

CRYSTALLINE COMPONENTS OF CALCULI

Table 1 summarizes the findings in 500 consecutive analyses of urinary calculi at the Bowman Gray School of Medicine. Stones which contain as much as 95 per cent by weight of a single crystalline structure are listed as "pure." The limitations of the qualitative chemical methods for analysis of stones have been clearly demonstrated by Prien and Frondel.[21] The crystallographic studies of these authors have added much to our knowledge of the crystalline components of urinary calculi but the internal structure and mechanisms of growth of the crystal lattice require further study. Many crystalline structures have empirical formulas which do not exist as molecular units in any other state. A single atom or ion is sometimes included in the formation of a crystal for no apparent reason other than to complete the particular crystal lattice. The mechanisms by which either adsorbed ions or units of structure are exchanged between the surface of crystals and the surrounding solution remain a controversial subject.[15] The bonds which maintain these units of the crystal lattice in their spatial relationships are so arranged that almost any large crystal, such as sodium chloride[8,17] or hydroxyapatite,[22] is in reality a single molecule.

TABLE 1
CHEMICAL COMPOSITION OF 500 RENAL AND URETERAL CALCULI

Primary Constituents	Per cent Total
Pure—95% by weight:	
Calcium phosphate	36.6
Calcium oxalate	11.2
Uric acid	3.4
Cystine	0.4
Mixed:	
Calcium phosphate and oxalate	44.2
Uric acid with phosphate and/or oxalate	4.2
	100.0

Secondary Constituents (not included above): Magnesium ammonium phosphate present in 12 per cent; traces of uric acid present in 8 per cent; only 6 per cent of calcium phosphate stones were free of other constituents.

The general rules relative to crystal formation and growth[8] have a limited applicability to the study of urinary concretions. Crystallization may be facilitated by nuclei which present sur-

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faces favorable to deposition of the solid phase. The component ions and atoms of a given crystalline substance tend to "fit" into the lattice structure on the surface of like crystals. Thus crystalline uric acid nuclei tend to grow by the addition of uric acid crystals. A basic unit of hydroxyapatite crystal[22] grows by additions of the exact quantities of calcium, phosphate and hydroxyl ions necessary to duplicate its crystal lattice. There is a preferential formation of large concretions, since a small mass is attracted to larger masses having relatively less surface area and consequently less total surface energy.

The fact that certain materials are available for crystallization in biologic solutions does not necessarily mean that they are insoluble, hence availability and solubility are not synonymous in this respect. However, both the availability for crystallization and the solubility of a given ion, atom or compound are influenced by a variety of forces. These include the hydrogen ion concentration, the buffer systems, the total ionic concentration, the relative electrical charges, the zeta potential, the adsorption of relatively insoluble units by highly soluble molecules and the formation of soluble and insoluble coordination or chelate compounds. For example, calcium in a solution of orthophosphates has entirely different properties from those which it possesses in a solution of condensed phosphates. It should be borne in mind, however, that the rules of physical chemistry are devised under carefully defined laboratory conditions, and that the application of one or two of these rules "out of context" to complicated biologic systems is usually impossible.

Solute Phase of Crystalline Components of Calculi. Urine is a buffered, ionic and molecular solution. It contains organic suspensoids of molecular weights up to seven million,[25] and inorganic units as small as the hydrogen ion. There is no rationale for comparing the solubility of these materials in water to their solubility in urine. Only confusion can result from such statements as "The urine is normally twenty times saturated with respect to calcium oxalate."[16] Urine is not normally supersaturated with respect to calcium ions, since a neutral salt of calcium can be titrated into a twenty-four-hour specimen of urine to double the average content of calcium without precipitation.[3] This same observation applies also to the oxalic acid radical.

Hypercalcinuria is not essential to the development of calculi.[7] The majority of our patients with renal calculi, but without evidence of such associated syndromes as multiple myeloma, hyperparathyroidism or renal disease, do not have an increase in the twenty-four-hour excretion of urinary calcium. Neither do the majority of patients with acidosis,[1] multiple myeloma, hyperparathyroidism[19] or thyrotoxicosis[2] have urinary calculi, even though increased excretion of urinary calcium is a consistent finding in these and other disease states.[1] Although the incidence of urinary calculi is much greater in such patients than in the population as a whole, it is apparent that hypercalcinuria *per se* is not the sole etiologic factor.

An increase in the quantity of soluble macromolecules or "protective colloids" in the urine cannot explain the absence of calculus formation in the majority of patients with hypercalcinuria. Removal of these colloids by ultrafiltration does not significantly influence the precipitation of the urinary salts.[3] Furthermore, the total quantity of colloids present in the urine of patients with calculous disease is much greater than in normal urine.[4] Some of these biocolloids are present in urine as a result of glomerular filtration while others are products of epithelial secretion. In general, the former are the proteins present in serum, and they carry into the urine with them their normal complement of bound calcium. Since the urinary pH is usually much lower than that of the plasma, these proteins will bind even less calcium in urine than in plasma. The urinary colloids derived from the epithelial secretions are largely composed of "uromucoid,"[5] which has a molecular weight of seven million.[25] The quantity of this component is much increased in the urine of all patients with calculous disease, and it is completely insoluble in urine of average concentration. There is thus no basis for the assumption that, because some colloidal solutions increase the solubility of calcium, the colloids of urine act in a similar manner.

Neither is there available any evidence that simple crystalluria has an etiologic relationship to the formation of concretions.[3] Crystalluria is a frequent clinical observation and may occur daily in the same individual for many months without the formation of a calculus.

On the basis of our present knowledge we cannot conclude that the formation of an organized calculus is solely, or even primarily,

the result of factors which influence the solubility products of the crystalline substances involved.

ORGANIC MATRIX OF CALCULI

Attempts to Recover the Matrix from Formed Calculi. We have been unable to determine accurately the quantity of organic material which may be considered to form the matrix of calculi. The usual methods for the dissolution of stones with strong mineral acids destroy the matrix and alter that which is recovered to form a gum-like mass of unidentifiable material. The partial dissolution of uric acid calculi in alkaline media has resulted in the leaching out of an organic matrix which contains all of the red-brown pigment characteristic of these stones. The undissolved residue is a soft, putty-like mass of white uric acid. The various colors observed in urinary calculi are an inseparable part of the organic matrix. All crystalline structures yet identified in urinary calculi[21] are normally white.

The most satisfactory method for demonstrating the presence of the organic matrix has been the slow chelation of the calcium ions with ethylene-diaminetetraacetic acid. Some of the organic material so obtained has shown metachromatic staining with aqueous toluidine blue.[14] The fact that the organic material recovered from various large calculi gives a positive Million test, a positive biuret test, a positive periodic acid test (Hotchkiss-McMannus stain) and a positive Molisch test indicates the presence of both protein and carbohydrate. In general, the stones composed primarily of the large, plane-surfaced crystals of calcium oxalate dihydrate have shown the least amount of matrix. The stones composed of mixtures of hydroxyapatite and calcium oxalate monohydrate have shown the greatest quantities of matrix.

None of these methods so far employed has resulted in recovery of a material suitable for complete analysis. Perhaps the recovery of the active mucoprotein matrix from formed calculi is no more feasible than the recovery of the clam's mantle from the nacreous lining of its dead shell.

Recovery of Materials from Urine Which May Form the Matrix of Calculi. A more practical approach may be directly toward a search for a "calcifiable matrix" in the urine of patients who are forming calcigerous urinary stones. A previously described technic[4] was used to recover all the non-diffusible components in twenty-four-hour specimens of urine from three normal subjects and from five patients with urinary calculi. Each residue was placed in a cellophane bag and dialyzed against distilled water until it was free of diffusible salts. These bags were then suspended in a sterile twenty-four-hour specimen of normal urine, which was stored at 1°C. to prevent bacterial growth. The sterile urine was replaced every seventy-two hours by a fresh specimen from the same normal subject. Layers of a firm crystalline material were observed to develop within the colloidal gel recovered from three of the patients with calculous disease, and smaller crystalline deposits were found in the samples from the other two patients with calculous disease. Analysis of these crystals revealed them to contain calcium and phosphate. No crystalline material could be distinguished by gross, microscopic or chemical analysis of the colloidal residues from the normal subjects. Figure 1 is a comparison of the crystalline residues from two patients with calculous disease and the uncrystallized residue from one normal subject. The greatest quantity of crystallization occurred in the colloidal residue from a patient who was subsequently demonstrated to have a functioning adenoma of the parathyroid gland. (Fig. 1A.)

Electrophoretic studies of the urinary proteins were made, and the urine of patients with calculous disease was found to contain a mucoprotein with electrophoretic mobilities different from that of any component of normal urine.[4] When this mucoprotein was suspended in calcifying solutions, there was precipitation of a calcium-mucoprotein chelate and the subsequent formation of a calcium phosphate precipitate.[5]

The aforementioned studies taken together were considered to be at least suggestive evidence that the urine of patients with calcigerous stones contains a "calcifiable matrix." No such material has been demonstrated in normal urine.

The urine of both normal subjects and patients with calculous disease was found to contain a mucoprotein of molecular weight 7×10^6. It is considered to be the principal product of the secretory activity of the urinary epithelium and has been referred to as uromucoid.[5] This material was independently isolated as the virus-inhibitory mucoprotein of urine by Gottschalk[12] of Australia, Odin[19] of Upsalla, and Tamm and Horsfall[25] of New York. It has a

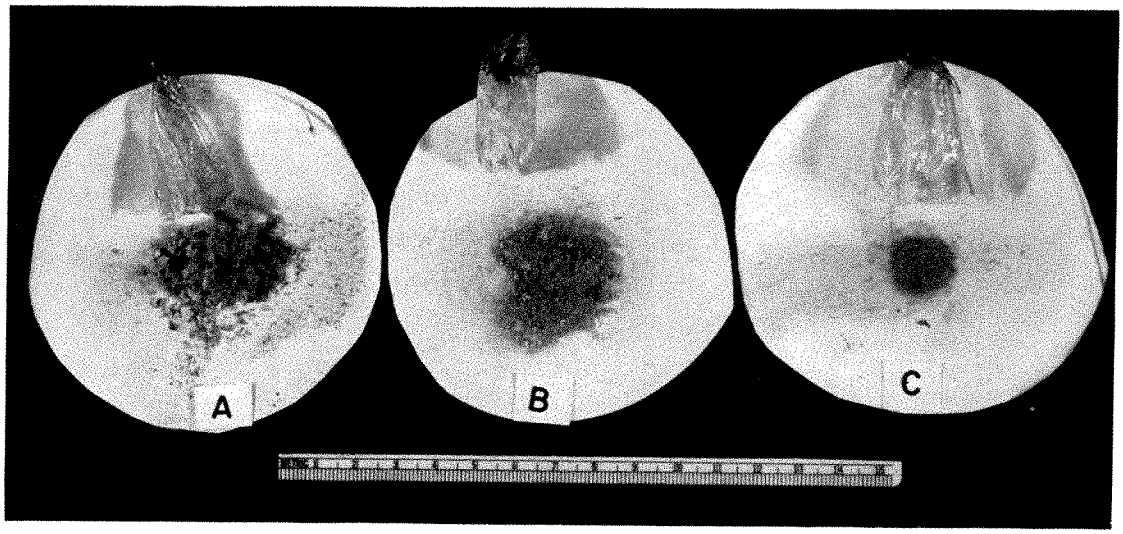


FIG. 1. Crystals containing calcium and phosphate which developed within the "calcifiable matrix" of colloidal material recovered from the urine of patients with growing calcigerous stones of the renal pelves. A, colloidal residue from twenty-four hour urinary output of a patient subsequently proven to have primary hyperparathyroidism. B, twenty-four hour colloidal residue from urine of patient with single renal calculus. C, twenty-four hour colloidal residue from normal control subject; no crystalline material developed in the control preparations. These colloidal residues were dialyzed free of salts and then suspended in cellophane bags in the same twenty-four hour urine specimen from a normal subject. Sterile urine was replaced at seventy-two hour intervals by twenty-four hour specimens from the same normal subject.

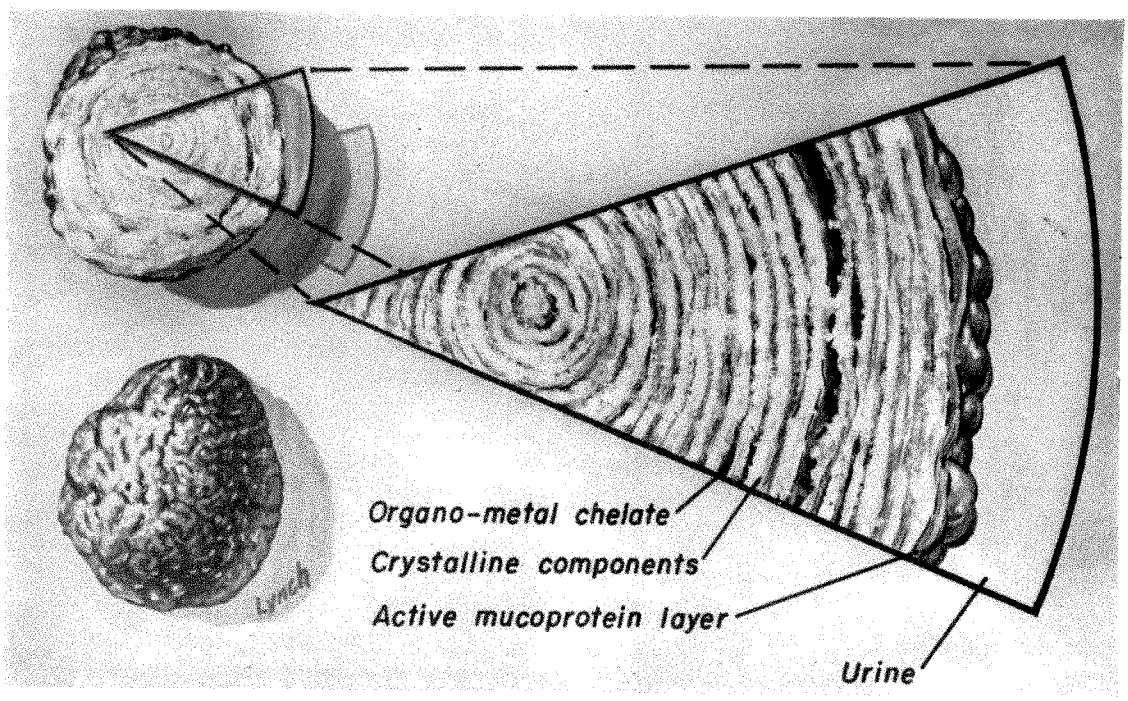


FIG. 2. Structure of a representative calculus removed from the renal pelvis. The center is hydroxyapatite; successive layers are mixtures of crystals of hydroxyapatite and calcium oxalate dihydrate. Dissecting binoculars were utilized for the detailed study.

total carbohydrate content of 25.9 per cent, composed of hexoses (galactose and mannose) 8.3 per cent, hexosamine 7.7 per cent, fucose 1.0 per cent and "sialic acid" 9.8 per cent. This material is quite insoluble in normal urine and in buffer solutions throughout the pH range of

This material was found to be soluble in buffer solutions of pH 12.3, which disrupted the molecule into smaller units with a molecular weight in the range of 3,710 to 4,250. These units were sufficiently similar in molecular weight and net negative charge to give electrophoretically homo-

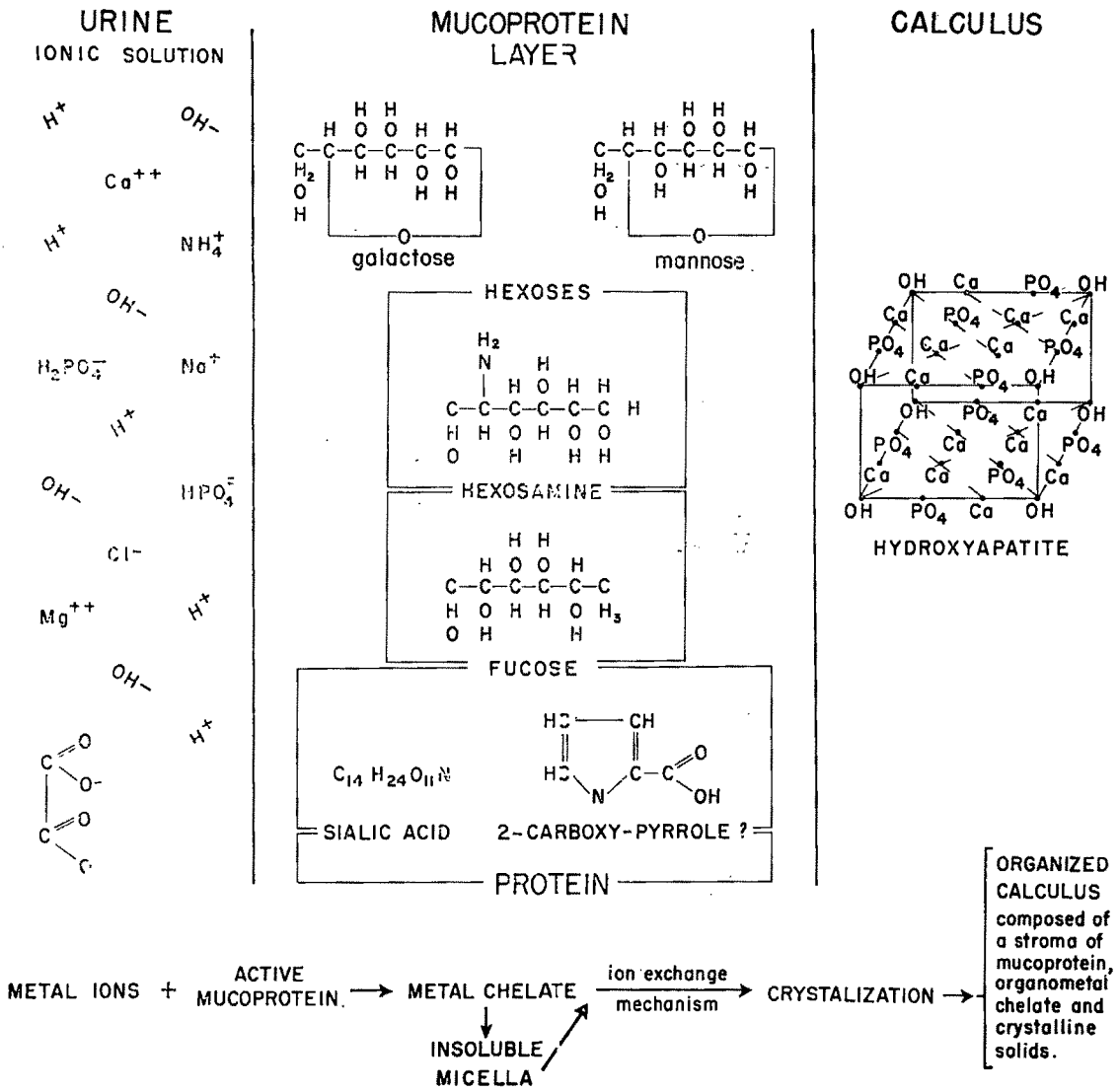


FIG. 3. Chemical composition of the various layers of the calculus illustrated in Figure 2. The basic units of the carbohydrate moiety of the urinary mucoprotein, uromucoid, are represented as the mucoprotein layer. No implications as to the quantitative relationships, structural bonds, optical isomerism or terminal groupings of these sugars are intended. There is evidence that uromucoid contains at least two carbohydrate moieties composed of slightly different combinations of these basic units. The calculus is represented as a basic unit of hydroxyapatite,[22] the most common crystalline component of calculi.

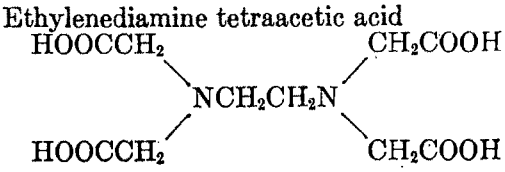
normal urine. When suspended in calcifying solutions, it did not produce any appreciable chelation of calcium as determined by conductometry.

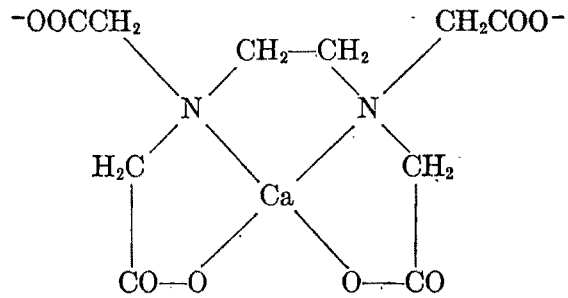
geneous patterns throughout the pH range of 4.5 to 12.0. Analysis of this material revealed a carbohydrate content of 21.5 per cent, composed of hexose 8.3 per cent, hexosamine 2.3 per cent,

fucose 2.1 per cent and "sialic acid" 8.8 per cent.[6] This soluble form of "uromucoid" was found to bind calcium to form a relatively insoluble calcium-mucoprotein complex. Conductometric and electrophoretic studies indicated this to be an insoluble mucoprotein chelate of calcium.[5]

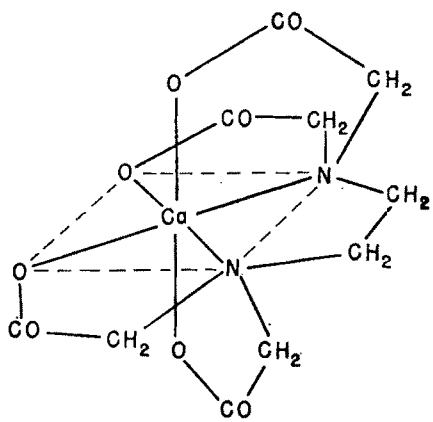
matrix. The exterior is covered with a glistening layer of mucoprotein, varying in thickness from a few hundred microns to several millimeters. Figure 3 is a schematic drawing of the general chemical composition of each of these layers.

Ethylenediamine tetraacetic acid

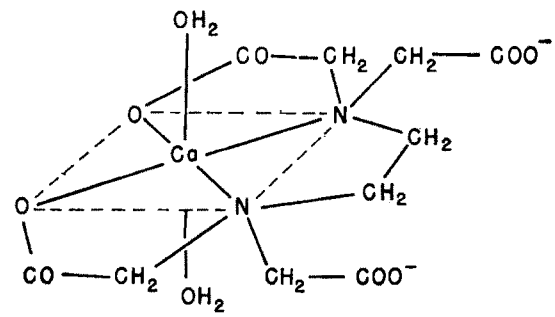




I Quadridentate



II Sexadentate



III Quadridentate

FIG. 4. Calcium chelates of ethylenediamine tetraacetic acid after Martell and Calvin.[17] III illustrates structural arrangement in which calcium with a coordination number of six may unite with a quadridentate chelate compound and still retain a bond relationship with hydroxyl or phosphate ions. Such a structure may be representative of an organic template which facilitates the crystallization of hydroxyapatite by the spatial arrangement of calcium, hydroxyl and phosphate ions.

The possibility thus exists that the "calcifiable matrix" present in the urine of patients with calculous disease is a modified or "activated" form of the uromucoid present in all urine.

INITIATION AND GROWTH OF URINARY CALCULI

Figure 2 is an illustration of the most usual type of renal calculus encountered in our series. The center is composed of calcium phosphate (hydroxyapatite) and the succeeding layers are a mixture of separate apatite and calcium oxalate crystals, with intermingled layers of organic

The carbohydrate fraction of the urinary mucoprotein contains substances which may form chelate compounds with calcium.[17] These include the hydroxy acids of galactose, mannose and fucose, glucosaminic acid and the proline structure, 2-carboxy pyrrole. Only the empirical formula of "sialic acid" has been determined[26] but inspection of this suggests that it probably represents a structure capable of chelate ring formation. (Figs. 3 and 4.) These carbohydrate units are selected from the analysis of "uromucoid;"[6,12,20] however, with the exception of 2-carboxy pyrrole, all of these components are

basic units of the carbohydrate moieties of serum mucoproteins.[26] For our present illustration it is thus irrelevant whether we ascribe these units to uromucoid or to a mucoprotein derived from the serum.

In any consideration of the solubility or ion exchange reactions of calcium and other metals, the chelate compounds are of peculiar importance. A metal chelate compound is a single molecule containing two or more electron donor groups which bind the metal ion to form an undissociated ring compound in which the metal is an integral unit. The divalent calcium ion has a coordination number of at least four and probably six. (Fig. 4.) A single quadridentate chelate molecule can thus tie up a calcium ion in three individual rings through four of these coordinate covalent bonds; or, conversely, a single calcium ion can tie up two molecules of a tridentate chelate compound, forming four rings through six bonds. Oxalic acid is an example of a simple bidentate chelate compound.

The physical and chemical properties of metal chelates are quite different from those of the metal salts, metal complexes or organometallic compounds. Calcium firmly caught in the grip of these chelate rings is no longer active in the same sense as ionized calcium in a similar solution. This fact is evidenced by alterations in electrical conductivity and electrophoretic properties, in solubility and in the production of color reactions, as well as a great many other properties.[17]

Although the metal ion is firmly trapped in the chelate ring system, it may be separated with extreme rapidity under certain conditions. Each organo-metal chelate has a definite stability constant, and a given metal can be readily displaced from the chelate structure by other metals higher in the series. The presence of competitive chelate structures, the hydrogen ion concentration, and a variety of diverse factors influence this separation of the metal from its chelate.[17] These properties have made the metal chelate compounds most valuable for removing lead from the human body,[23] providing soluble minerals for plant nutrition and separating complex mixtures of metal ions.

Most of the metal chelate compounds studied to date have their greatest usefulness in the formation of highly soluble metal chelates. The mucoproteins of urine are characterized by having the chelate rings firmly attached to a protein moiety, and the calcium chelates thus formed tend to be highly insoluble macromolecules.[5] The significance of this property in the formation of urinary concretions is immediately apparent. An active chelate compound of this type can form an insoluble calcium-mucoprotein molecule by chelating calcium from urine which contains even minimal quantities of calcium. If this complex remains adherent to the walls of the urinary tract, a very high local concentration of calcium may be built up in normal urine. Thus a colloidal micelle[13] of extremely high calcium content may develop.

Under the influence of shifts in hydrogen ion concentration, the presence of oxalic acid, orthophosphates or particularly condensed phosphates, or other changes in environment,[17] the chelate rings may be induced to release calcium, with the resultant local formation of crystalline calcium compounds. In the initial stages these crystals may form adjacent to the mucoprotein or within its meshes; but as the process repeats itself many times, the successive layers of crystal units will tend to form on the surface of the pre-existing crystal. The structure may thus become a calculus, with the layers oriented as shown in Figure 2.

As successive layers of crystalline material are laid down upon the surface of the calculus, variable quantities of the mucoprotein layer may be left entrapped to form the "matrix." However, the point of growth of the stone is at the surface of the crystalline layer and immediately beneath the surface of the mucoprotein cover. Thus the growth of calculi may be likened mechanically to the growth of the oyster's shell as the mantle lays down successive layers of nacre.

COMMENT

It is apparent that the mucoprotein responsible for this transfer of ions from urine to crystalline solid must be different from any found in normal urine, and we have used the term "activated mucoprotein" to denote this difference. We have been unable to demonstrate any such activity in any of the proteins found in normal urine.[5] If "uromucoid" is the responsible mucoprotein, its structure must be modified in some manner to free the carboxyl and amino groups which are probably responsible for calcium chelate formation. It seems unlikely that hydrogen bonds alone could tie up these groups to the exclusion of calcium, since the stability constants of most such structures are

considerably less for hydrogen than for calcium. It is more likely that some internal arrangement of the carbohydrate moiety prevents the chelation of calcium until the structure has been rearranged or disrupted. The observation that uromucoid is a much more active chelating agent for calcium after its molecule has been degraded and a portion of the hexosamine content split away lends support to this latter concept.

A coating of normal uromucoid might be expected to form a protective inert layer which could limit the growth of the crystalline components of calculi. Such a layer of inactive normal uromucoid may account for the molding of branched calculi to conform to the outlines of the epithelium-lined renal pelvis and calyces. The tendency for "staghorn" calculi to fill the renal pelvis rapidly and then remain quiescent may also result from this contact with the secretions from relatively normal epithelium.

We have frequently observed clusters of the large plane-surfaced crystals of calcium oxalate dihydrate on the surfaces of calculi such as the one shown in Figure 2. These deposits may be the result of crystallization of this material on a large surface without the intervention of a chelate mucoprotein step. However, at least half of the "pure" calcium oxalate stones listed in Table I have a discernible nucleus of calcium phosphate. It is probable that the remainder have a microscopic nidus of calcium-mucoprotein chelate, with or without phosphatic crystals. The occurrence of magnesium ammonium phosphate crystals in calculi may represent a similar type of "secondary deposit" or surface crystallization; however, chelate agents for calcium will also chelate magnesium if the local conditions are favorable and if all available calcium has been previously bound.

SUMMARY

Considerable evidence has accumulated which would indicate that the organic matrix of urinary calculi plays an active part in the local concentration and precipitation of calcium. This metal is the principal cationic unit in the crystalline structure of more than 90 per cent of all urinary concretions. It is suggested that the abnormal (activated) mucoproteins of urine[5] chelate calcium from solution to form relatively insoluble micelles[13] of colloidal calcium-mucoprotein units. This is the initial step in calcigerous stone formation and is followed by crystallization within the meshes of the mucoprotein micella. These same mucoproteins, forming a surface layer, may produce a local concentration of calcium on the surface of the growing concretion. They may even serve as an organic template to facilitate the spatial arrangement of calcium and hydroxyl ions[17] in the growth of the apatite lattice.[22]

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Seminars on Carbohydrate Metabolism

The Metabolism of Carbohydrates*

A Review

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THE present paper is intended to give background information in the area of mammalian carbohydrate metabolism. Similar contributions from other sources are expected to appear subsequently in this Journal treating with endocrine regulation and clinical disturbances of sugar metabolism. It is our intent to supply the necessary information relating to the biochemical reactions of the common sugars as these are currently understood. This will permit the future development by other contributors to this series of such matters as the regulation of carbohydrate metabolism by endocrine mechanisms and the metabolic defects in the diseases of carbohydrate metabolism.

The benefits which the mammalian cell derives from its nutrients may be divided into two classes. (1) All of the compounds necessary for its continued survival and the performance of its normal function must come to the cell from the nutrient directly or must be synthesized from precursors which are supplied in this nutrient. Included in this category is the need for continuous replacement within the architecture of the cell of those structural components which are being destroyed. (2) All of the energy required for the continued survival and operation of the cell must be derived from the breakdown of energy-containing compounds of the nutrient.

The present discussion of carbohydrate metabolism will be oriented toward a consideration of the compounds which the cell can form from the sugars which are supplied to it and of the sources of the energy and the mechanisms of its release or storage in the course of carbohydrate catabolism.

Photosynthesis and the Origins of Biochemical Energy. The prime mover with respect to the energy of biochemical reactions is the phenomenon of photosynthesis. Ultimately, all the energy with which we are concerned is derived from that portion of the solar spectrum that is absorbed by chlorophyll. In green plants this dependence is fairly direct; in animals this dependence, though less immediate, is equally real. Photosynthesis may be defined as the reductive fixation of carbon dioxide into organic molecules with the liberation of oxygen gas, brought about by the action of light:

$$H_2O + CO_2 \xrightarrow{\text{light}} 1/n\ \underset{\text{(carbohydrate)}}{(CH_2O)_n} + O_2\uparrow \quad (1)$$

This process has been bisected into two major steps, only the first of which is photodependent:

$$H_2O + \underset{\text{(electron acceptor)}}{X} \xrightarrow{\text{light}} H_2X + \underset{\text{(active oxygen)}}{[O]} \rightarrow \tfrac{1}{2}\ O_2\uparrow \quad (2)$$

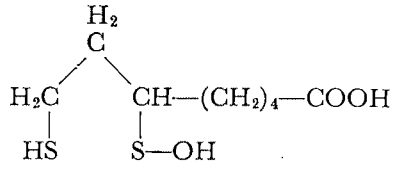
In this, the so-called "light" reaction, water is cleaved in the presence of an electron acceptor, X, to yield a reduction product, H_2X, and oxygen.

By means of this photo-energized reaction the energy of certain electrons is enormously en-

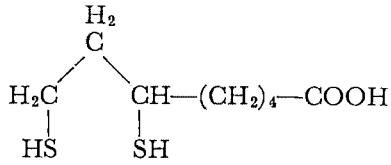
* From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education and Welfare, Bethesda, Md.

hanced as they are delivered, in a single step, from the stable, low energy condition which they occupy in water to a high energy condition which they occupy in the reduced cofactor H_2X. The fixation of CO_2 is a secondary process not, *per se*, photodependent.

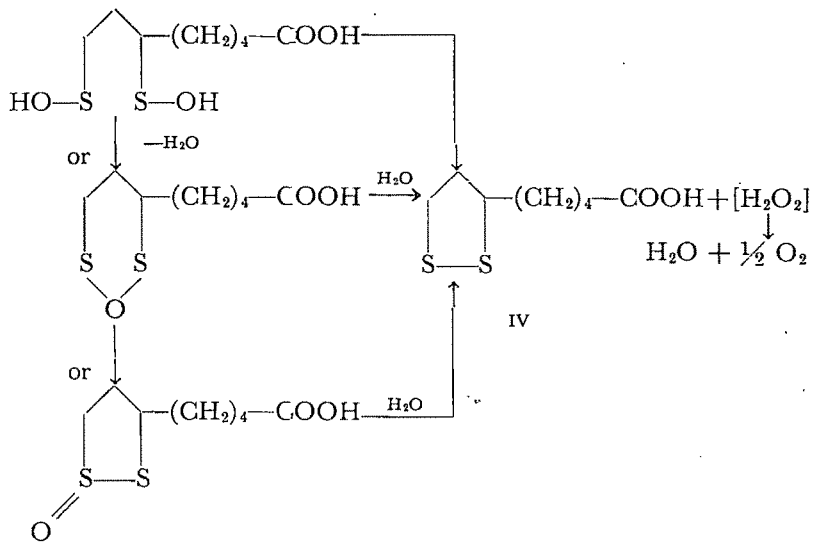
During the catabolic, exergonic phase, which occurs in green plants when the light is turned off and in mammalian cells at all times, the energy of electrons cascades downward, not in a single step which would be explosive and wasteful but in many small discrete steps until the basal energy level of water is again achieved. The generation of CO_2, like its fixation, is a secondary process involving the decarboxylation of keto acids formed incidentally by the various oxidative processes.



Two molecules of this product then dismute to yield (III):

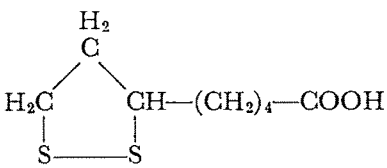


which is H_2X (equation *2*), and a product (IV) which may be termed the active oxygen (equation *2*):



(*3*)

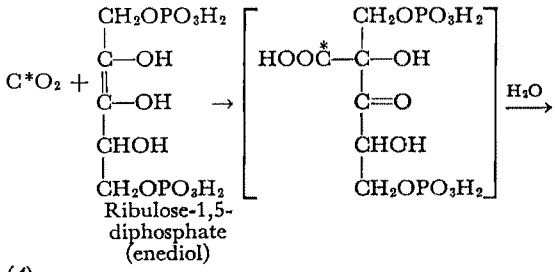
Recently, the probable nature of X in equation *2* has been elucidated. It is lipoic acid, having the structure (I):



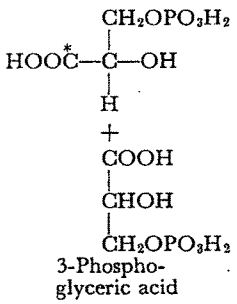
The essential role of this compound in the oxidation of pyruvic acid and its entry into the Krebs tricarboxylic acid cycle had been recognized long before its structure was known. When chlorophyll is excited by light it is pictured as passing energy on to lipoic acid, the strained five-membered ring of which reacts with water to yield a thiolsulfenic acid of the type (II):

The net result of this total process is to decrease the concentration of the cyclic lipoic acid (I). Since this compound is essential for oxidation, via the Krebs cycle, of pyruvic acid, herein lies the probable explanation for the fact that during photosynthesis the Krebs cycle in plants is essentially non-operative.

As indicated earlier, the fixation of carbon dioxide is itself not photodependent but is linked to the reductive pressure which stems from the photosynthetic generation of reduced coenzymes. The probable initial steps of CO_2 fixation by green plants include condensation with the ketopentose, ribulose-1,5-diphosphate, and subsequent cleavage of the product to yield 2 molecules of 3-phosphoglyceric acid:



(*4*)



The location of the radioactivity initially incorporated by green plants photosynthesizing in an atmosphere of $C^{14}O_2$ has been shown to be in the carboxyl carbon of 3-phosphoglyceric acid (v):

$$H_2O_3PO—CH_2—CHOH—C^{14}OOH$$

The continued operation of this reaction requires the regeneration of five-carbon sugars and essential for this is the reduction of phosphoglyceric acid to the triose phosphate, 3-phosphoglyceraldehyde (vi):

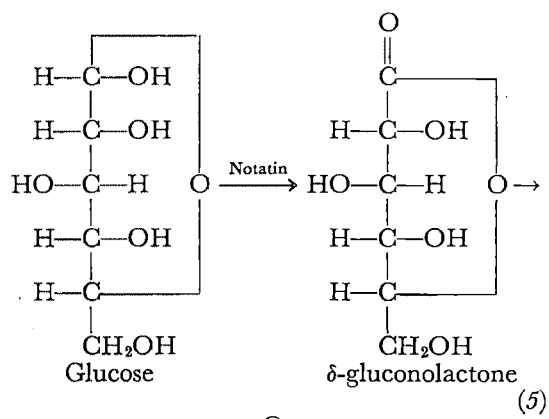
$$H_2O_3PO—CH_2—CHOH—CHO$$

Whereas the immediate source of hydrogen for this reduction is probably reduced diphosphopyridine nucleotide, DPNH, the ultimate source of hydrogen is doubtlessly reduced lipoic acid (iii).

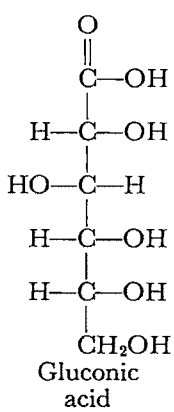
The triose phosphate generated serves as a source of hexoses such as glucose by reversal of the Embden-Meyerhof pathway, which is to be discussed in the next section. It also permits regeneration of the pentose, ribulose, required in reaction *4*. The details of these routes are beyond the scope of this review but it may be mentioned that a prominent pathway for pentose formation is the reversal of a portion of the transketolase pathway, described on page 102.

Glycolysis via the Pathway of Embden and Meyerhof. For the energy that is contained in the glucose molecule to become available to the mammalian cells which consume it, the bonds holding the molecule together must be broken. In the respiring organism the final products of such breakdown are generally CO_2 and H_2O. It has been found that this process occurs over fairly well defined pathways, some of which are common to a variety of cells and tissues. Of these the most studied is that referred to as the Embden-Meyerhof pathway, a series of reactions whereby glucose is degraded to lactic or pyruvic acid or both. Among the many fates of pyruvic acid is its introduction into the citric acid cycle of Krebs, whereby it is finally oxidized to carbon dioxide and water. However, since the citric acid cycle is common to metabolites of lipids and amino acids as well as to those from carbohydrate sources, it will not be considered as falling within the area of this review.

The reaction which initiates the Embden-Meyerhof sequence, and probably the most commonly occurring of the metabolic fates of glucose in the mammal, is phosphorylation in position 6. Non-phosphorylating fates of glucose do occur in microbial biochemistry and the best known example of these is the notatin reaction:



(*5*)



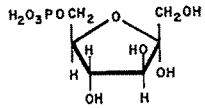
A by-product of this reaction is hydrogen peroxide, which is apparently the product responsible for the bactericidal action of notatin. A number of years ago a similar reaction was discovered in mammalian liver wherein glucose was dehydrogenated by DPN to yield gluconolactone. However, the significance of this reaction in the glucose economy of the intact mammal is questionable.

Glucose phosphorylation is a ubiquitous reaction in animals and in microbes. The unique source of phosphate is adenosine triphosphate, and the enzyme is termed hexokinase. This enzyme occupies a key position in glucose catabolism and was the enzyme implicated by Cori as related to the mode of action of anterior pituitary extracts and of insulin. Essentially all cells of higher animals have the capacity to phosphorylate glucose, but hexokinases from various sources differ in their specificities with respect to the sugar component. Some, such as intestinal mucosa hexokinase, effect phosphorylation of galactose and fructose as well as glucose, while others, such as a particular hexokinase of muscle and liver, operate solely upon fructose to give fructose-1-phosphate. The over-all reaction

(6) 

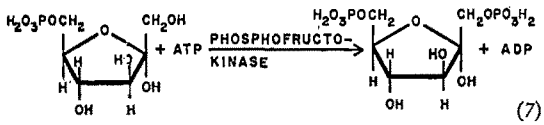
is exergonic and essentially irreversible. One mole of energy-rich phosphate in the form of ATP is expended for each mole of glucose phosphorylated.

The product, D-glucose-6-phosphate, has four important fates open to it: (1) Under the influence of a specific glucose-6-phosphatase it may be irreversibly hydrolyzed to yield glucose and inorganic orthophosphate. This reaction does not occur in muscle but is known to occur in liver and probably also occurs in kidney, intestinal mucosa and other tissues. It is the immediate source of the bulk of the biosynthetic blood glucose. (2) Glucose-6-phosphate may be transformed to glucose-1-phosphate. This reaction and its consequences are discussed on page 106. (3) Glucose-6-phosphate may be oxidized to 6-phosphogluconic acid. The implications of this reaction appear on page 102. (4) Glucose-6-phosphate may be reversibly isomerized to fructose-6-phosphate (VII):



The enzyme for this transformation is termed phosphoglucose isomerase and the product is on the main path of the Embden-Meyerhof sequence. This same product may also arise from fructose directly by phosphorylation with ATP and fructokinase of yeast, brain or muscle.

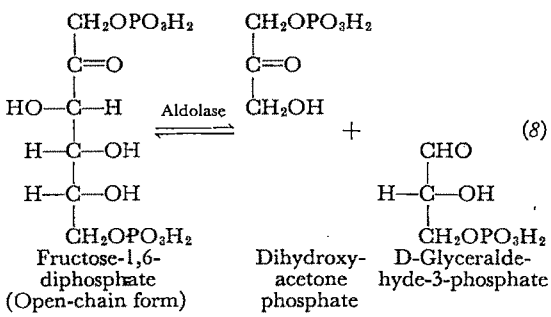
Further phosphorylation of fructose-6-phosphate consumes a second mole of energy-rich phosphate and yields fructose-1,6-diphosphate as a product:



(7)

This reaction, like the hexokinase reaction, is exergonic and irreversible. There does, however, appear to be a phosphatase capable of catalyzing the hydrolysis of fructose diphosphate to fructose-6-phosphate and inorganic phosphate.

The next reaction in the Embden-Meyerhof sequence is a reversible cleavage of the bond between the two central carbon atoms 3 and 4 of the hexose skeleton:

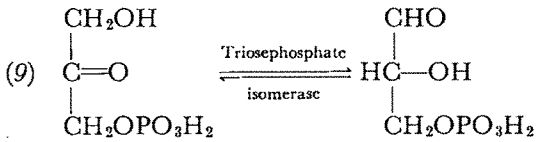


(8)

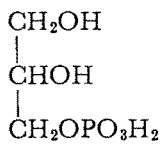
The products of this reaction in the catabolic sense are the phosphoric acid esters of the ketotriose, dihydroxyacetone, and the aldotriose, glyceraldehyde. In the synthetic or anabolic

sense, the enzyme aldolase is remarkably specific with respect to dihydroxyacetone phosphate but a variety of aldehydes have been found to replace glyceraldehyde-3-phosphate, leading to the possible synthesis of a variety of sugars. The synthetic possibilities of this enzyme have only recently been generally appreciated.

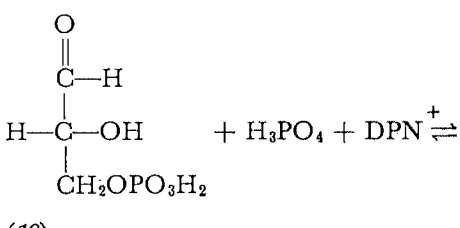
The two triose phosphates are readily interconvertible in the presence of triosephosphate isomerase and are often considered as an equilibrium mixture:



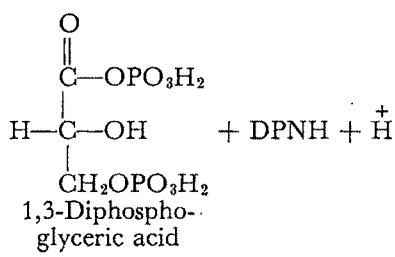
Dihydroxyacetone phosphate is of interest as the source of glycerol and α-glycerolphosphoric acid (VIII)



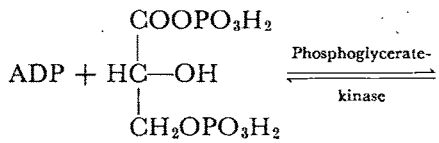
to which it is readily reduced by DPNH, and these products undoubtedly participate in the synthesis of neutral fats. Glyceraldehyde-3-phosphate is subject to a remarkable oxidation involving the obligatory participation of inorganic phosphate. The details of this reaction, which involves a sulfhydryl enzyme and oxidized diphosphopyridine nucleotide, are currently unclear but the over-all stoichiometry is expressed by the equation:



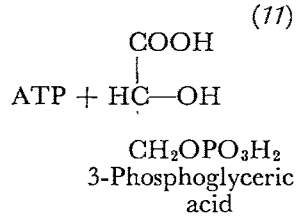
(10)



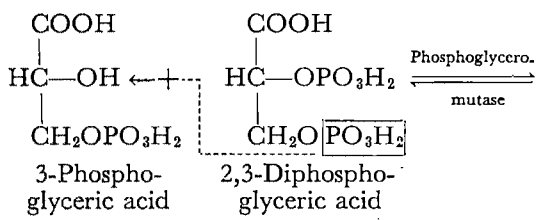
This reaction is unusual in two important regards. In the first place, it is the sole step intervening between glucose and pyruvic acid in this sequence which involves oxido-reduction. Secondly, it is a reaction whereby inorganic orthophosphate is consumed while an energy-rich phosphate bond, —C(=O)—OPO_3H_2, is generated. The phosphate in position 1 of the product is of sufficiently high energy to enter the adenosine triphosphate system:



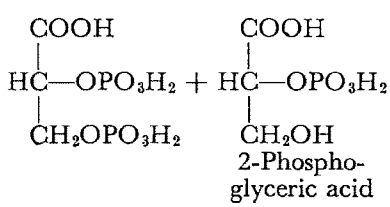
(11)



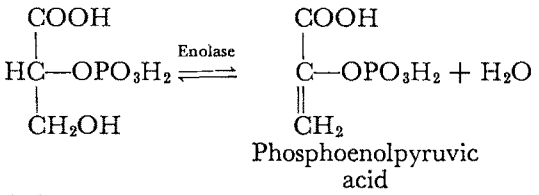
The product of this reaction is in equilibrium with its isomer, 2-phosphoglyceric acid, and since the reaction is accelerated by the presence of 2,3-diphosphoglyceric acid a mechanism analogous to that of the phosphoglucomutase reaction (page 106) has been postulated:



(12)

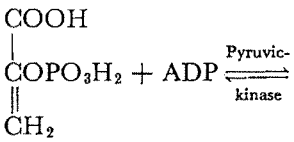


In the presence of enolase, 2-phosphoglyceric acid loses a mole of water:

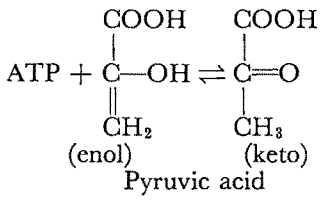


(*13*)

to yield the phosphoric acid ester of (enol) pyruvic acid. Phosphate in this situation is also rich in energy and capable of entering the ATP system:



(*14*)



In the course of its normal operation in respiring tissues a large part of the pyruvic acid formed undergoes oxidative decarboxylation to yield acetyl-coenzyme A. This important product is a precursor for synthesis of fatty acids, sterols, and so on, and for CO_2 via the Krebs tricarboxylic acid cycle. Pyruvic acid is also an acceptor of amino groups to yield alanine and an acceptor of CO_2 to yield oxaloacetic acid. When oxygen is unavailable the reduced DPNH formed in the phosphoglyceraldehyde dehydrogenase reaction reduces pyruvic to lactic acid, which then is the stable final product of so-called "anaerobic glycolysis." With normal oxygen tensions in mammalian tissue, very little lactic acid accumulates and pyruvic acid may be considered to be the terminus of the Embden-Meyerhof sequence.

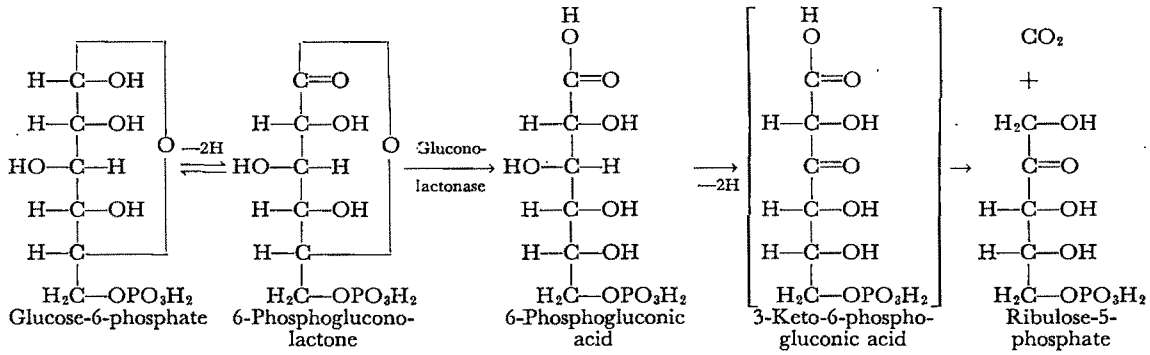
The gross energy yield liberated when a mole of glucose is broken down to two moles of lactate is approximately 58,000 calories, which is a small fraction of the energy yield, 686,000 calories, of the total oxidation of glucose to CO_2 and H_2O. In the course of the process some energy is conserved, as the following accounting of high-energy phosphate (~P) will reveal:

	~P Yield
glucose → glucose-6-phosphate	−1
fructose-6-phosphate → fructose-1,6-diphosphate	−1
2(diphosphoglyceric acid) → 2(phosphoglyceric acid)	+2
2(phosphopyruvate) → 2(pyruvate)	+2
Yield: moles ~P per mole glucose	+2

The Embden-Meyerhof pathway, with minor modifications, appears to operate in all mammalian tissues. It is the sole recognized pathway of glucose breakdown in muscle and is a major pathway in many other tissues, such as brain.

Glucose Oxidation via 6-phosphogluconic Acid. Whereas the pathway of Embden and Meyerhof has, until recently, been considered as the prototype which, subject to minor modifications, described the utilization of glucose, growing evidence for the existence of a distinct and alternative pathway has accumulated in the past few years. This route has variously been called the "hexose monophosphate shunt," the "direct oxidative pathway" and the "Warburg-Lipmann-Dickens pathway" but is perhaps most appropriately termed the "phosphogluconate oxidation pathway." It stems, as does the Embden-Meyerhof pathway, from glucose-6-phosphate and is initiated by a series of oxidative steps. See formula (*15*), page 102.

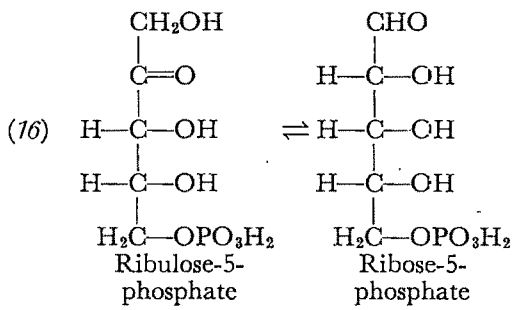
In each of the two oxidative steps triphosphopyridine nucleotide serves as hydrogen acceptor. The enzyme for the first reaction of the series, glucose-6-phosphate dehydrogenase, was named "Zwischenferment" by Warburg many years ago. The second enzyme, 6-phosphogluconate dehydrogenase, is believed to oxidize the sugar at carbon atom 3 but the 3-keto acid, the postulated product, has not been definitively characterized. Carbon 1 of glucose is then



(*15*)

eliminated as CO_2 yielding a pentose, ribulose-5-phosphate.

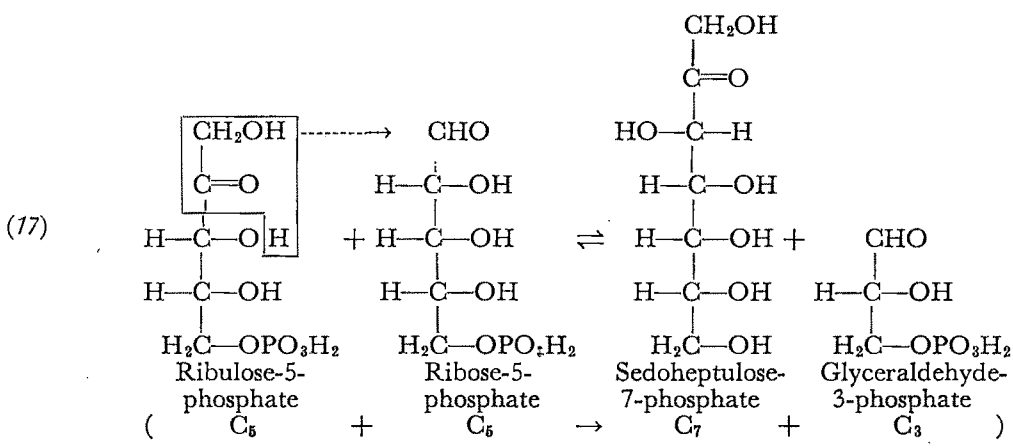
The action of a phosphopentose isomerase, acting analogously to phosphohexose isomerase (page 104), brings the ketopentose and the aldopentose into equilibrium:



(*16*)

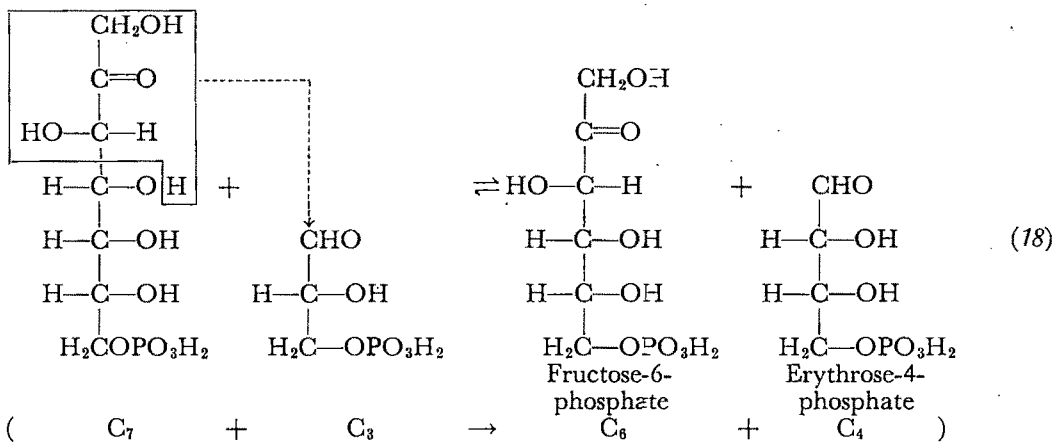
Ribose-5-phosphate may be activated at position 1 by reaction with ATP to yield 5-phosphoribose-1-pyrophosphate, and this product serves as a source of pentose in the synthesis of ribose nucleotides and ribose nucleic acids. Alternatively, ribose-5-phosphate is subject to transformation to ribose-1-phosphate by a mutase mechanism comparable to that in the hexose series and this derivative may also serve as a source of pentose in the synthesis of ribose nucleosides.

Carbon atoms 1 and 2 of ribulose are quite reactive. In the process of photosynthesis it is this pair of carbon atoms which ultimately accepts CO_2 from the atmosphere to yield phosphoglyceric acid. This pair of carbon atoms is of interest to us in another respect. The enzyme transketolase can, in the presence of thiamine pyrophosphate, transfer these carbon atoms, in the form of enzyme-bound glycolaldehyde ($HOCH_2CHO$), from ribulose-5-phosphate to certain acceptors among which is ribose-5-phosphate:



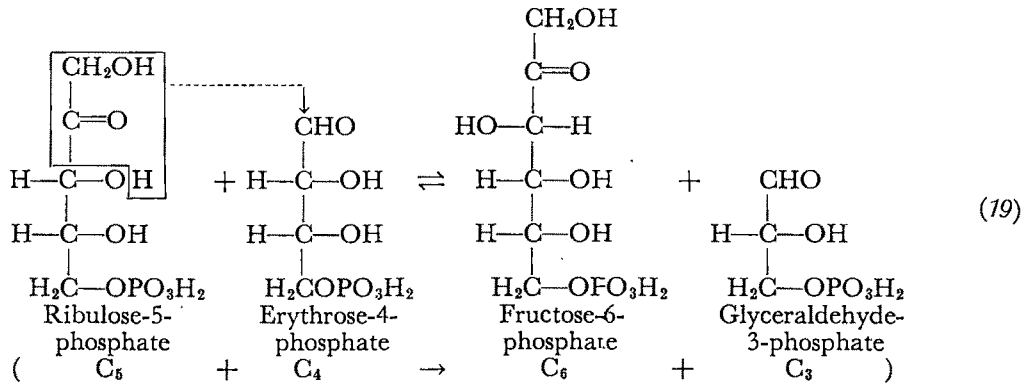
(*17*)

The seven-carbon sugar, sedoheptulose phosphate, may in turn react with the three-carbon sugar, glyceraldehyde phosphate, in a transaldolase reaction involving transfer of the top 3 carbon atoms from the former to the latter:



(*18*)

The metabolic relations of fructose-6-phosphate have already been discussed (page 99). The four carbon sugar fragment may serve, much as ribose-phosphate does, as an acceptor of active glycolaldehyde in a transketolase reaction:



(*19*)

In considering the cyclic operation of this series of reactions one additional fact should be borne in mind, namely, that glyceraldehyde-3-phosphate, by reversal of the steps of the Embden-Meyerhof sequence, may be converted to hexose ($C_3 \rightarrow \frac{1}{2}\ C_6$). Summation of these several steps reveals the following:

$$
\begin{array}{rl}
 & 3\ C_6 \rightarrow\ 3\ C_5 + 3\ C_1\ (CO_2) \\
 & 2\ C_5 \rightarrow\ C_7 + C_3 \\
C_7 + & C_3 \rightarrow\ C_6 + C_4 \\
C_5 + & C_4 \rightarrow\ C_6 + C_3 \\
 & C_3 \rightarrow\ \tfrac{1}{2}\ C_6 \\
\hline
\text{Sum:} & 3\ C_6 \rightarrow 2\tfrac{1}{2}\ C_6 + 3C_1
\end{array}
$$

In other words 3 molecules of CO_2 are generated from 3 molecules of glucose with the potential capacity for regeneration of $2\frac{1}{2}$ molecules of hexose.

It is interesting to compare and contrast this pathway with the Embden-Meyerhof pathway. In the phosphogluconate oxidation pathway, beginning with glucose-6-phosphate, no ATP is required but an obligatory oxidation occurs early in the reaction sequence. Repeated operation of the cycle as delineated yields CO_2 as the sole and final product, with release of all the energy introduced into the glucose molecule by photosynthesis. Concerning the disposition and possible conservation of this energy, there is no definitive information. The Embden-Meyerhof pathway, on the other hand, when beginning with glucose-6-phosphate, consumes ATP in the formation of hexose diphosphate. If lactic acid is the accumulated end product, no net oxidation has occurred and the sequence can and does proceed anaerobically. Terminating at lactic acid, this sequence releases only a fraction of the energy contained in glucose.

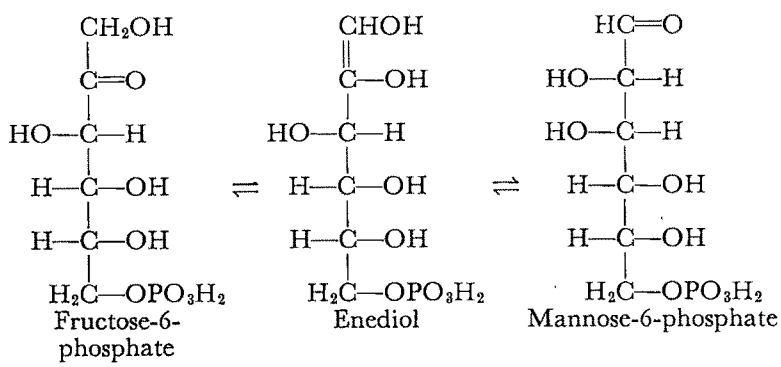
The steps of the phosphogluconate oxidation pathway were, in many cases, first studied in microbial systems. However, there is good reason to suppose that the same or very similar steps also occur in certain mammalian systems. Whereas skeletal muscle appears to catabolize glucose exclusively over the steps of the Embden-Meyerhof pathway, the phosphogluconate oxidation pathway has been shown to operate in liver, mammary gland, testis and certain other tissues.

It would be erroneous to conclude that the two pathways described here for glucose catabolism necessarily are the only pathways that occur. Other routes have been indicated but the evidence in support of their occurrence in mammalian systems is thus far lacking.

Hexose Interconversions. In addition to the various pathways for glucose catabolism involving rupture of the hexose carbon skeleton, there are means available to the organism whereby it can convert glucose into other hexoses with retention of the intact carbon chain. Such reactions lead to the formation of certain monosaccharides which are required for the biosynthesis of lactose and mucopolysaccharides, and to others which are necessary for detoxication mechanisms.

The interconversion of glucose-6-phosphate and fructose-6-phosphate catalyzed by phosphoglucose isomerase has been described on page 99. Mannose, which differs from glucose in its configuration about carbon atom 2 and is found in nature as a constituent of various polysaccharides and of glycoproteins, can be formed as its 6-phosphate ester from fructose-6-phosphate in a reaction catalyzed by a phosphomannose isomerase present in muscle:

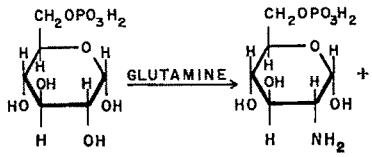
(20)



Fructose-6-phosphate — Enediol — Mannose-6-phosphate

Mechanistically, this reaction, which is similar to that for the interconversion of glucose- and fructose-6-phosphates, involves the intermediary formation of an enediol, and consequently the open forms of the sugars are directly implicated. It should be noted that fructose-6-phosphate is an obligatory intermediate between glucose- and mannose-6-phosphates.

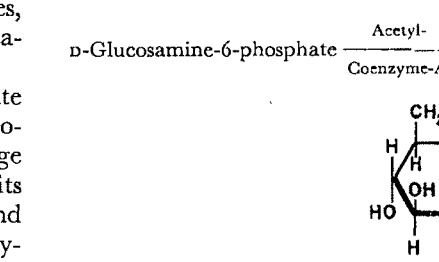
The synthesis of glucosamine, which occurs as its N-acetyl derivative chiefly in polysaccharides of the tissues, was first demonstrated with an extract of Neurospora mycelia:



(21)

D-Glucose-6-Phosphate — D-Glucosamine-6-Phosphate

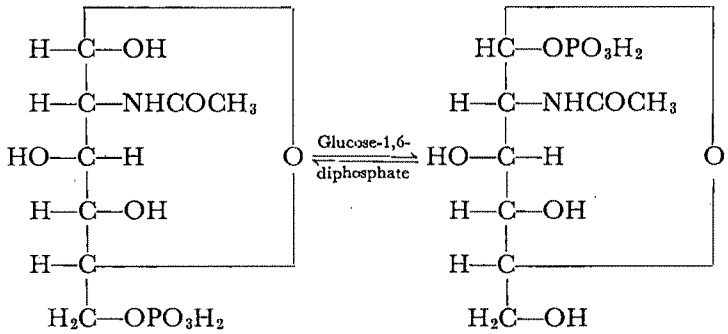
At least two further enzymatic reactions intervene between the formation of this product and the incorporation of the glucosamine moiety into mucopolysaccharides:



(22)

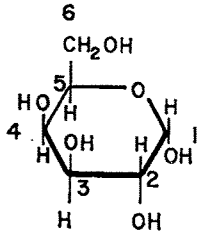
N-acetyl-D-glucosamine-6-phosphate

The acetylated material is then isomerized to the 1-phosphate in a manner reminiscent of the phosphoglucomutase reaction discussed on page 106.

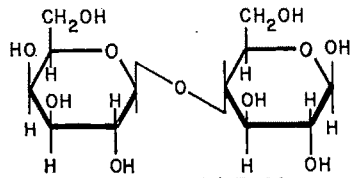


N-Acetyl-D-glucosamine-6-phosphate ⇌ N-Acetyl-D-glucosamine-1-phosphate (*23*)

Another aldohexose of biochemical interest is D-galactose (IX), which differs from glucose in its configuration about carbon atom 4.

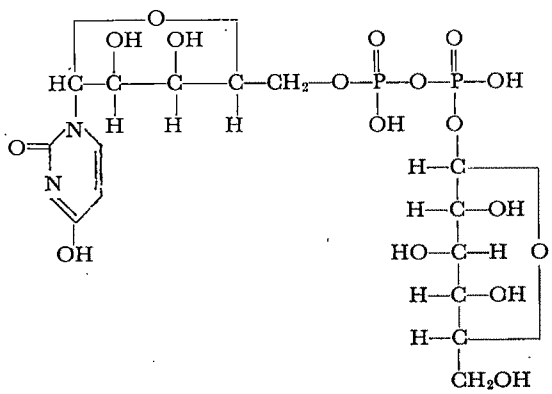


This sugar is a component of several polysaccharides, and of the disaccharide lactose (X), a sugar peculiar to mammals.



4-(β-D-Galactopyranosido)-D-Glucopyranose
β-D-Lactose

Actually glucose-1-phosphate and galactose-1-phosphate are interconvertible, that is, inversion about carbon atom 4 is reversible in the presence of the enzyme phosphogalactose isomerase. The coenzyme for this reaction is uridine diphosphoglucose, UDPG (XI),



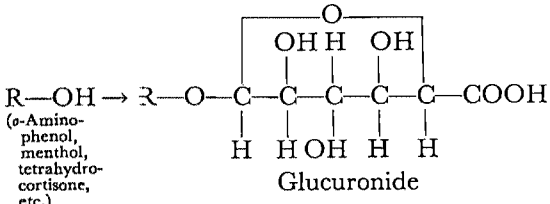
and the following steps have been shown to occur:

Glucose-1-phosphate + Uridine triphosphate (UTP) ⇌ UDPG + Pyrophosphate

UDPG ⇌ UDPGalactose (Phosphogalactose isomerase; UTP) ⇌ Galactose-1-phosphate (*24*)

Little is known of the mechanism of this isomerization other than the fact that it occurs without rearrangement of the carbon skeleton. It should be noted that galactose-1-phosphate can also arise in liver from galactose and ATP by a galactokinase-catalyzed reaction.

Another series of conversions which has been recently elucidated involves the formation of glucuronic acid and its conjugation with certain alcohols to form glucuronides:



(*25*)

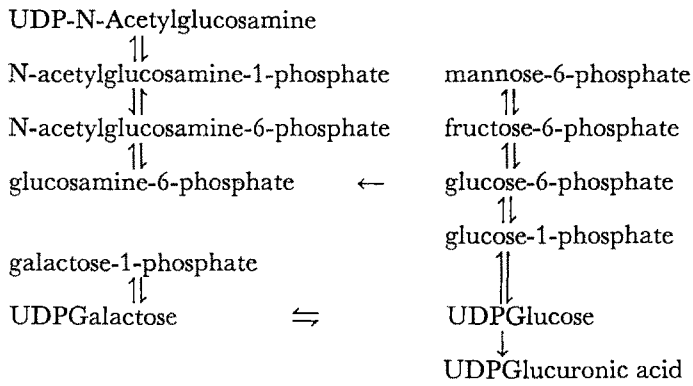
Products of this type of detoxication reaction are excreted in the urine. The oxidation of carbon atom 6 of glucose to give the uronic acid again involves the remarkable coenzyme UDPGlucose and a DPN-linked enzyme derived from liver:

UDPGlucose + 2 DPN^+ →

UDPGlucuronic Acid (UDPGA) + 2 DPNH + $2H^+$ (*26*)

"Active" glucuronic acid, UDPGA, then acts as the glucuronide donor in the synthesis of the conjugates. This reaction, which is catalyzed by an enzyme derived from the microsomes of liver, has been demonstrated in the formation of glucuronides of *o*-aminophenol, menthol and tetrahydrocortisone. Although demonstration of the specificity of the enzyme system involved must await purification of the microsomal preparation, the glucuronide conjugating reaction involving UDPGA appears to be a general mechanism for the formation of such phenolic and alcoholic derivatives.

A summary of relationships of certain hexoses is shown in the following diagram:

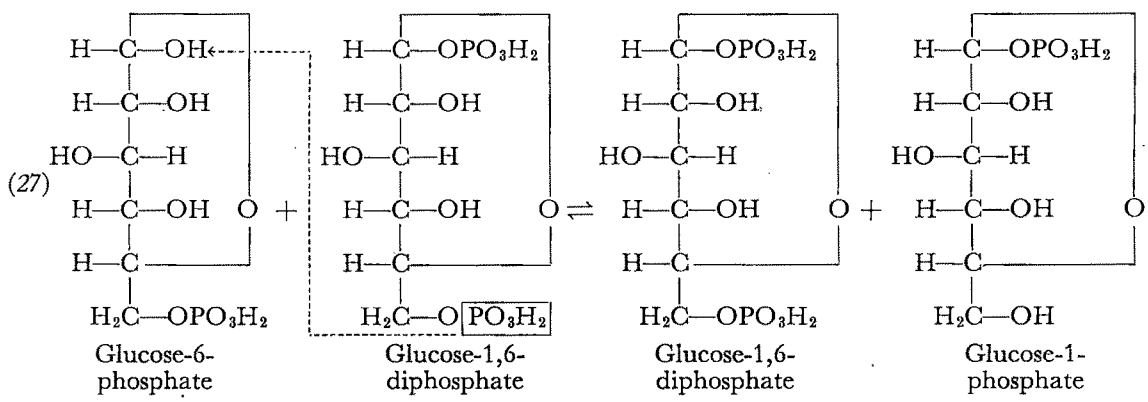


vested especially in the liver, to synthesize glucose from non-carbohydrate organic molecules. The other is the ability of various tissues to store glucose, whether of dietary or synthetic origin, as polysaccharide. This ability, very widespread in nature, results in the accumulation of starches in vegetable cells and of glycogen in animal cells.

The phenomenon of glycogen storage is a happy one in several regards. The molecule of glycogen is of enormous molecular size and of low intrinsic viscosity, hence contributes minimally to the viscous or osmotic properties of the intracellular fluid. Sugar stored as glycogen is readily available for mobilization and indeed such mobilization, and coincident deposition of hexose phosphate, occurs continuously in the normal animal. The unique source of all glycogen, so far as is known, is glucose-1-phosphate. This compound is generated in the body in an enzyme-catalyzed transphosphorylation:

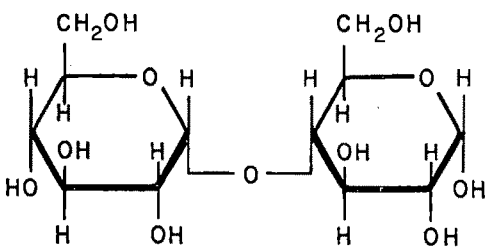
Glycogen and the Mechanism of Carbohydrate Storage. In view of the intermittent nature of ingestion of food, two devices exist in the mammalian organism to satisfy the continuous need of certain tissues, such as the central nervous system, for glucose. One of these is the capacity,

(27)

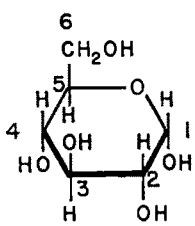


The reaction is catalyzed by the enzyme, phosphoglucomutase. Since glucose-1,6-diphosphate is both a reagent and a product of the reaction, and since its concentration does not diminish as the reaction proceeds, it need be present only in catalytic amounts. In fact, since glucose-1,6-diphosphate was a minor contaminant of impure mutase preparations, the requirement for this reagent escaped detection for many years.

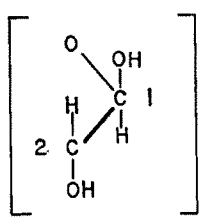
Activation of the aldehydic carbon of glucose, as by phosphorylation, appears to be a prime requisite for the biologic establishment of glucosidic bonds. A glucoside may be defined as a derivative of glucose substituted in the number 1 or aldehydic carbon atom. In the family of compounds under present discussion, the substituent is itself a glucose residue, and the parent of this family is the glucose-glucoside, maltose (XII):



With reference to the numbering of the carbon atoms of glucose drawn in this convention the oxygen bridge will be recognized as extending from carbon-1 of one glucose to carbon-4 of its neighbor:

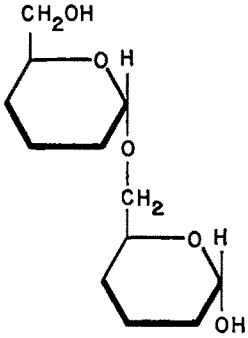


α-D-Glucopyranose

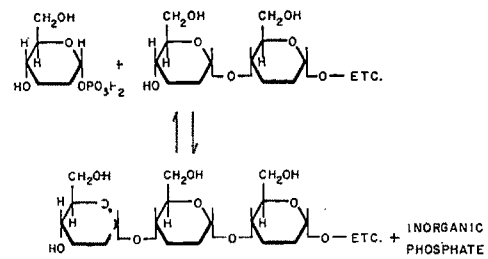


(β-D-)

The so-called α-1,4′ bond is the sole glucosidic bond of the starch, amylose. In amylopectin, and to a greater extent in glycogen, another glucosidic bond exists, the α-1,6′ bond, exemplified in isomaltose (XIII):



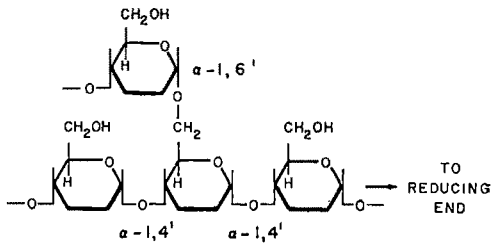
The primary step whereby the organism generates glycogen involves a phosphorolytic condensation between glucose-1-phosphate and the maltosidic end group of a pre-existing polysaccharide molecule:



(28)

This reaction is freely reversible, its equilibrium being determined by the relative concentrations of glucose-1-phosphate and inorganic phosphate. The enzyme catalyzing this reaction is phosphorylase, which has been isolated from animal as well as from vegetable sources. Under certain circumstances phosphorylase may become inactivated and then readily reactivated by addition of adenylic acid. Epinephrine favors this reactivation in the case of liver or muscle phosphorylase whereas glucagon is believed to assist in the reactivation of the liver enzyme.

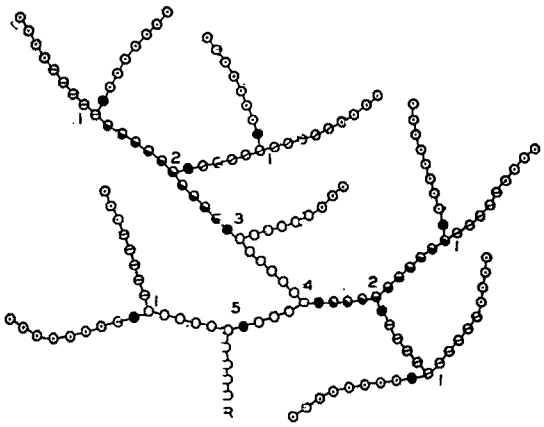
The expected product of the phosphorylase reaction, generating solely α-1,4′ glucosidic bonds, would be long unbranched chains of glucose residues, each with a single reducing (aldehydic) end and a single non-reducing end. This is indeed the case with amylose. In glycogen, however, there are numerous non-reducing ends to each molecule and the molecule is highly branched. A branch point exists wherever a glucose residue bears substituents on carbon atoms 1, 4 and 6:



Phosphorylase is incapable of generating α-1,6′ bonds but a second enzyme, a "branching" enzyme, has been described which is a transglucosidase. It apparently catalyzes a reaction wherein glucose residues are transferred from α-1,4′ to α-1,6′ linkage, the new linkage being formed at the expense of the old. The enzyme has been named amylo-(1,4 → 1,6)-transglucosidase and has been shown to occur both in liver and in muscle.

The combined action of phosphorylase and the branching enzyme results in the generation of highly branched polysaccharides of an arboreal nature as shown in Figure 1. The branching patterns of liver and muscle glycogens differ somewhat, probably a consequence of difference in the relative activities of the participating enzymes. The basic scheme of polysaccharide storage is remarkably uniform throughout the animal and vegetable kingdoms.

Closely associated with the synthesis of glycogen is its enzymatic breakdown. In the gastrointestinal tract the breakdown of polysaccharides of the starch-glycogen type is initiated by the hydrolytic cleavage of α-1,4′ glucosidic bonds by the catalysis of α-amylases of the saliva and pancreatic juice. α-Amylase also exists in the blood and possibly in the solid tissues of the body but its role in intracellular glycogenolysis is obscure. Better understood is the phosphorolytic cleavage of α-1,4′ bonds, under the influence of phosphorylase, a reaction which is the precise reversal of that previously shown (reaction *28*). The attack by inorganic phosphate liberates the terminally situated glucose residue



R, reducing end of molecule.
⊙, ⊖, ⊜, glucose units removed by the first, second, and third digestions with phosphorylase, respectively.
●, glucose unit split off as free glucose from 1,6 linkage by amylo-1,6-glucosidase.

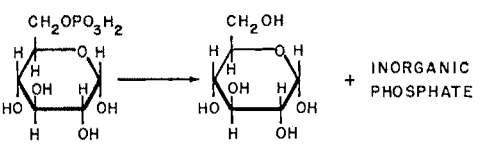
FIG. 1. The structure of glycogen. (From Larner, J., Illingworth, B., Cori, G. T. and Cori, C. F. *J. Biol. Chem.*, 199: 647, 1952.

as glucose-1-phosphate. This product, by reversal of the phosphoglucomutase reaction (reaction *27*), yields glucose-6-phosphate; the glucose residue of glycogen thereby is reintroduced into the main stream of glucose metabolism.

The attack by inorganic phosphate and phosphorylase upon glycogen begins at the unbranched, non-reducing ends of the glycogen molecule. (Fig. 1.) When a branch point is approached and a glucose residue in α-1,6′ linkage is exposed the action of phosphorylase ceases. This product, refractory to further attack by phosphorylase, is termed a phosphorylase limit dextrin. It can be converted into a phosphorylase-sensitive polysaccharide once again by the removal of the exposed glucose residues in α-1,6′ linkage and this is effected hydrolytically by an enzyme present in both liver and muscle, amylo-1,6-glucosidase, the "debranching" enzyme. Just as glycogenesis

from glucose-1-phosphate is a result of the combined action of phosphorylase + branching enzyme, so glycogenolysis is a consequence of the combined action of phosphorylase + debranching enzyme. Kinetic studies of glycogen turnover in the intact animal have revealed that all these processes are continuously operating in liver and in muscle. Glycogen molecules are gaining peripherally situated glucose residues (phosphorylase reaction) and at the same time new branch points are being established (branching enzyme). Since these processes occur even though the nutritional state and the glycogen content of the tissues are sensibly constant, the steps of glycogen breakdown must be pictured as accompanying those of glycogen synthesis.

A few generalizations about glycogen reserves are appropriate. In terms of weight, and more strikingly in terms of calories, the glycogen reserves of the mammal are small compared with the reserves of depot fat. The glycogen reserves of the liver are readily depleted by various procedures, including fasting, thyroid intoxication, hypoxia, etc. The glycogen stores of the muscle, although not exhausted by fasting, can be effectively eliminated by convulsions. Epinephrine administration is followed, possibly as a consequence of phosphorylase activation, by an initial depletion of glycogen. This is most obvious in the liver but in the previously fasted animal is also apparent in the muscle. Rapid breakdown of glycogen in liver may be accompanied by a rise in blood glucose due to the presence, in this organ, of a specific glucose-6-phosphatase:



(29)

GLUCOSE-6-PHOSPHATE GLUCOSE

This enzyme is lacking in muscle, hence the only free glucose which arises when muscle glycogen breaks down is that liberated by amylo-1,6-glucosidase. Most of the biologic derivatives of muscle glycogen must by glycolyzed to pyruvic or lactic acids before they enter the blood stream.

Abnormally large accumulations of glycogen may be induced experimentally or may occur pathologically. In death due to insulin shock, enormous quantities of glycogen, especially in muscle, may be found, particularly if convulsions are avoided. Glycogen storage disease has been analyzed by Cori and in most cases its pathogenesis can be attributed to lack of glucose-6-phosphatase in liver or to abnormalities in branching pattern of the polysaccharide.

Other Polysaccharides of Biologic Importance. Dextrans, which are polyglucosides wherein the α-1,6′ linkage is the dominant one, have recently assumed practical importance in view of their use as plasma volume expanders. These polysaccharides are formed from sucrose by certain microorganisms including Leuconostoc dextranicum. The reaction involves transglucosidation by an enzyme called dextransucrase:

$$\underset{\text{(Sucrose)}}{\text{n Glucosylfructoside}} \rightarrow \underset{\text{(Dextran)}}{(\text{Glucosyl})_n} + \text{n fructose} \qquad (30)$$

A host of polysaccharides are known which, in contrast to glycogen, starch, cellulose, dextran, etc., yield more than one sugar product upon total hydrolysis. These are called heteropolysaccharides. Polysaccharides which are comprised of roughly equimolar amounts of glucuronic acid and either acetylglucosamine or acetylgalactosamine occur in connective tissues. Thus hyaluronic acid contains a repeating disaccharide unit of glucuronyl-acetylglucosamine in which the bonds are of the β-1,3′ variety. Heparin apparently has a similar structure with the additional feature of sulfuric acid residues attached along the chain. Chondroitin sulfate, the characteristic polysaccharide of cartilage, is composed of glucuronic acid, acetylgalactosamine and sulfuric acid.

The blood group substances as well as the specific soluble sugars of many bacteria belong to the class of heteropolysaccharides. Many of these have very complex structures and a variety of monosaccharides have been recovered from the products of their hydrolyses.

SUMMARY

Any attempt to separate the metabolism of carbohydrates from that of fats or of amino acids is at best arbitrary. Many metabolic pathways are now known to be shared in common between various groups of tissue constituents, many intermediates may be derived from non-carbohydrate as well as carbohydrate precursors.

In the composition of the present report the authors have limited the subject matter to the metabolic reactions of those compounds which

the organic chemist would call "sugars." On this basis, and also on the basis that it is as much a part of fatty acid or amino acid metabolism as of carbohydrate metabolism, discussion of the tricarboxylic acid cycle has not been included. An attempt has been made to review the current state of our knowledge of the origins of the common sugars, their catabolic fates, their metabolic transformations and the devices employed in their biologic storage.

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Combined Staff Clinic

Obesity

THESE are stenotyped reports of Combined Staff Clinics of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Gilbert H. Mudge.

DR. JOHN V. TAGGART: At a time when major areas of the world face a chronically inadequate food supply it is highly significant that one of our leading nutritionists, Dr. F. J. Stare, can state that "an excessive caloric intake is the single, most extensive nutritional problem affecting public health in this country." If moderate obesity is defined as a 20 per cent excess of weight over the so-called ideal, it can be estimated that 15 million people in this country are so affected. However, to classify obesity strictly in terms of a comparison with some hypothetic ideal is undoubtedly misleading in many instances. From a medical standpoint we might better consider it as that degree of corpulence which interferes seriously with an individual's well-being or efficiency.

What are the ways in which obesity threatens the individual's well-being? Insurance statistics appear to provide tangible evidence in this matter. These have been summarized succinctly in the often repeated phrase of Louis Dublin that, "The penalty of overweight is a one-quarter to three-quarters increase in mortality." For example, in a group of persons between forty-five and fifty years of age mortality rates increase about 1 per cent for each pound excess weight. But mortality statistics alone cannot give us an adequate picture of the penalties of overweight. We know, in addition, that the obese person is more susceptible to diabetes, cardiovascular disease and cholelithiasis, that he is accident-prone and that he carries an increased risk when subjected to surgery. Perhaps even more important is the fact that excessive weight imposes a burden on day to day labor, often to the extent that work itself becomes a trial. Finally, and perhaps most serious of all, the obese individual, though he may realize no physical discomfort, may suffer most in his personal relationships.

Much of this Clinic will be devoted to a consideration of the etiologic and physiologic aspects of obesity. Let me state that we have implicit faith in the validity of the first law of thermodynamics. Many novel ways of evading this law have been proposed in the past but we must regard them as emotional and groundless. It follows, therefore, that any accumulation of tissue, adipose or otherwise, represents a positive caloric balance. The problem of obesity is largely one of balance between appetite and energy expenditure. That this balance is maintained normally by one of the most delicately poised homeostatic mechanisms merits emphasis. The majority of individuals can maintain a fairly constant weight over a period of months or years without making any conscious adjustment of their food intake to match a widely fluctuating energy expenditure. Why then are some individuals able to stabilize at an acceptable weight while others fail to do so?

First, we know that attitudes toward food vary tremendously and that these attitudes stem in part from geographic, ethnic and economic considerations. For the individual who comes from an area where food is in short supply, food becomes of tremendous importance. We know also that there are definite familial patterns of eating. The habits of eating established during childhood are perhaps among the most potent and lasting influences affecting our daily activities. Secondly, it should be noted that psychologic factors may be of paramount importance in some cases of obesity. This, too, has been aptly summarized by the late Cyril Connolly who said, "Obesity is a state of mind, a disease brought on by boredom and disappointment." While we do not wish to minimize the importance of psychologic factors in the etiology of obesity, this aspect of the problem will not be our major concern today. It is our hope that a

review of the dynamics of body weight may lead to a better understanding of the mechanisms which underlie the adjustments of appetite to metabolic needs. Our first speaker, Dr. Theodore B. Van Itallie of St. Luke's Hospital, has kindly consented to summarize for us the many important experimental contributions which have been made in this field during recent years.

DR. THEODORE B. VAN ITALLIE: For many years a large proportion of physicians and nutritionists has held that obesity is a simple matter of overeating. Like all tautologies, this thesis is logically incontestable. Yet, it evades the essential question: Why do obese persons overeat?

A decade ago, when Newburgh[1] reviewed the subject of human obesity from the physiologic standpoint, he concluded that, although many painstaking investigations had been made, no abnormal metabolic process had been disclosed to account for the accumulation of excess fat in obesity. Similarly, Conn[2] concluded that the mechanisms for dissipating energy from the obese body usually show no abnormality and that obesity must therefore result from an excessive desire for food.

The judgments of Newburgh, Conn and others that obesity in man is unaccompanied by metabolic abnormalities have tended to discourage clinical investigation into the etiology of obesity at the physiologic level; however, there have been advances in our knowledge of intermediary metabolism and in physiology and psychology which have thrown further light on the problem of obesity. As a result of the work of many investigators in these fields it is possible to set up a tentative scheme in which the various factors concerned with the regulation of food intake and fat storage may be integrated. By means of such a model it is possible to indicate some of the sites at which disorders of this "regulatory system" might be expected to occur.

A precise definition of obesity always must be arbitrary; however, Keys and Brozek[3-4] have properly emphasized that obesity in man is not necessarily determined by the simple measurement of body weight but is best demonstrated by a consideration of body composition. Nor is it completely accurate to say that all forms of obesity represent only an imbalance between energy intake and energy expenditure. This is usually the case but there are exceptions in which the body retains abnormal quantities of fat even after prolonged undernutrition.[5] Moreover, as Conn[2] has pointed out, endocrine influences may affect distribution of body fat without appreciably altering its total content.

Etiologically, there seem to be two general categories of obesity: those which are due primarily to energy imbalance, whether due to excessive intake or decreased expenditure, and those which are due primarily to abnormalities in intermediary metabolism. Such abnormalities may involve either excessive fat synthesis or impaired fat degradation. In one instance there is an excess of substrate without an accompanying metabolic disorder; in the other, there is an abnormality in the way substrates are handled by cells leading, in turn, to an increased energy intake.

TABLE I

TENTATIVE GROUPING OF FORMS OF OBESITY ACCORDING TO MECHANISM

I. Regulatory obesity (No primary metabolic abnormality)
   - A. Psychologic
     1. Neurotic overeating
     2. Non-neurotic overeating (cultural dietary pattern)
   - B. Physiologic
     1. Increased intake—hypothalamic disorder
     2. Decreased output—forced immobilization

II. Metabolic obesity
   - A. Enzymatic—? genetic obesity in mice
   - B. Hormonal—hyperadrenocorticism
   - C. Neurologic—? lipodystrophy (autonomic)

For obesities which result primarily from disorders of energy balance without evidence of metabolic abnormality, the general term regulatory obesity might be employed. For obesities which represent primarily disorders affecting fat turnover, the term metabolic obesity seems appropriate. (Table I.)

Regulation of Energy Intake. As a background for discussion of the various forms of obesity some sort of physiologic framework is indispensable. Although there is still a great deal to be learned about the mechanism of food intake regulation, it is known that there are two important centers concerned with hunger and satiety in the ventral hypothalamus. Each of these centers is bilaterally symmetrical, and electrolytic lesions of the more medial pair (in the ventromedial nuclei) result in hyperphagia, impaired satiety and obesity.[6] Appropriate lesions lateral to the ventromedial nuclei are followed by complete cessation of feeding. Animals with such lesions will not consume food

even if it is placed in their mouths.[7] Thus there appears to be a feeding center and a regulatory center. When the regulatory center is injured, feeding becomes uncontrolled; when the feeding center is damaged, feeding stops or is impaired. Whether the hyperphagia which follows destruction of the ventromedial nuclei results directly from interruption of some direct neural connection between the two centers or indirectly as a result of metabolic changes secondary to such destruction is not yet clear. There are autonomic and metabolic changes associated with destruction of the ventromedial nuclei which of themselves could lead to increased food intake.[8–9]

In a recent review Brobeck[10] has discussed three theories which attempt to explain the regulation of food intake. One theory proposes that animals eat for calories and that some mechanism exists for signaling the intake of a certain amount of potential energy. For example, Adolph[11] has stated that, "Food acceptance and the urge to eat in rats are found to have relatively little to do with a 'local condition of the gastrointestinal canal,' little to do with the 'organs of taste' and very much to do with quantitative deficiencies of currently metabolized materials." How the animal can measure potential energy is not known and, as Brobeck points out, no change in composition of body fluids or in pattern of intermediary metabolism directly proportional to energy intake has been identified. A recent study by Strominger, Brobeck and Cort[12] appears to exclude the caloric content as the intrinsic food factor producing the change in an animal which signals satiety.

A second possible mechanism is one proposed by Brobeck himself. His explanation is based on the fact that in the assimilation of food animals exhibit a rise in total heat production. This phenomenon has been termed by Rubner the specific dynamic action (S.D.A.). Brobeck has suggested that as extra heat is released from food it alters the conditions of regulation of body temperature and the animal stops eating to prevent hyperthermia. Thus, according to Brobeck, animals "eat to keep warm."

In support of Brobeck's hypothesis is the finding of Booth and Strang[13] that the rise in skin temperature after eating has the same course in time as that of the S.D.A. and correlates well with the onset of satiety. Moreover, when the composition of the diet is changed to alter the concentration of protein, carbohydrate, fat, or the total bulk, rats will adjust their intake so that the calculated S.D.A. varies less than any other known factor of the diet.[14] And, of course, it is well known that the hypothalamus contains temperature-sensitive cells.

The "thermostatic" theory described by Brobeck has several questionable features. For example, the mechanisms ordinarily employed by the body to dispose of extra heat and to maintain temperature are so effective that it seems superfluous to invoke hunger and satiety as additional thermoregulatory devices. There is no evidence to suggest that hot food *per se* has a greater satiety value than the same amount of food served cold, or that a hot bath destroys one's appetite. It is evident that energy is needed for the performance of work as well as for the maintenance of body temperature. Hence the concept of "eating to keep warm" appears to neglect the fact that any theory of food intake must account for the conditioning effect of changing energy requirements upon energy intake. No relationship has been identified between S.D.A. and energy requirements.

A third theory of food intake listed by Brobeck is the "glucostatic" theory of Mayer and his associates.[15–17] Briefly, this theory postulates that satiety occurs during active uptake of blood glucose by appropriate receptors in the central nervous system and that the desire for food is felt only when the supply of blood glucose to the "glucoreceptors" is curtailed. Thus, from the glucostatic view, the quantity of carbohydrate available in the body at any one time exerts a regulatory effect on food intake.

Brobeck's major criticism of the glucostatic theory has been that the existence of glucoreceptors in the central nervous system is unproved.[10] Actually, there is little doubt that such glucoreceptors exist. Duner[18] has recently demonstrated that there is a center in the hypothalamus sensitive to changes in local concentration of glucose. When the glucose concentration in the hypothalamic region is selectively elevated inhibition of epinephrine secretion by the adrenal medulla follows. This effect is mediated via the splanchnic nerves. A number of investigators have shown that lowering the blood sugar by means of insulin stimulates gastric peristalsis.[19] If the vagi are sectioned this effect is abolished. Conversely, raising the blood glucose level may result in inhibition of gastric peristalsis,[20–21] although

intravenous administration of amino acids does not appear to affect the motility of the stomach in fasting dogs.[22] Hence it seems probable that there are centers in the hypothalamus responsive to changes in blood glucose and capable of initiating and inhibiting gastric contractions, and of influencing the rate of epinephrine secretion by the adrenal.

Larsson (and Forssberg)[23] recently reported studies on the metabolism of the hypothalamic region which contains centers known to be concerned with the regulation of food intake. They measured radiophosphate incorporation and the hydrolyzable phosphate content (creatine phosphate and energy-rich terminal groups of adenosine triphosphate) in three hypothalamic areas (A, B and C) of fed and fasted rats. Their analyses disclosed that in the area of the hypothalamus containing the lateral and ventromedial nuclei (C) significantly more P^{32} was taken up in hungry than in fed animals. The others, control (A and B) areas, of the hypothalamus did not display such increased phosphate retention during hunger and, indeed, showed less P^{32} uptake in the fasted than in the fed state. The livers of hungry rats were distinguished by an increased uptake of radiophosphate; however, blood, muscle and cerebrum as a whole showed no significant differences in phosphate incorporation when the fasting and fed states were compared from this standpoint. The concentration of energy-rich phosphate was markedly higher in the C-samples than in the control hypothalamic areas of hungry rats. The opposite was found in fed rats.

These findings are consistent with the view[14] that the hypothalamic areas concerned with food intake may be more characteristic of peripheral cells in their metabolic properties than they are of brain tissue. For example, if they behaved like nervous tissue in their uptake of glucose they would be unable to signal impending depletion of available carbohydrate since nervous tissue uses carbohydrate at a more or less constant rate regardless of whether the organism is starved or fed.[24]

Peripheral arteriovenous glucose differences appear to serve as better indexes of the availability of blood glucose to body tissues than either arterial or venous levels alone.[16] Large arteriovenous glucose differences, properly interpreted, correlate well with satiety; however, further confirmatory studies are needed before it can be stated with assurance that such an association has etiologic significance. A correlation also has been described between small arteriovenous glucose differences and desire for food. (Fig. 1.)[21]

The hypothalamus is connected by neural pathways to the cerebral cortex; there are also efferent fibers which travel from the hypothalamus to the gastrointestinal tract. A connection exists between the hypothalamus and the fat depots: the sympathetic nerves, which innervate adipose tissue, link with neurons originating in the hypothalamus.[25] If the hypothalamus is visualized as subject to influences from the cerebral cortex as well as from the circulating blood and sensory nerves from the periphery, the importance of so-called psychosomatic factors in the scheme of food intake regulation becomes intelligible. Stunkard[26] has shown that talk about food with patients cannot elicit gastric hunger contractions if active utilization of carbohydrate (as inferred from peripheral arteriovenous glucose differences) is taking place. Gastric hunger contractions can be elicited if peripheral arteriovenous glucose differences are small. On the other hand, certain emotionally disturbed patients, notably schizophrenics, will deny that they are hungry even when powerful gastric contractions are being recorded on the kymograph. Thus it would appear that physiologic and psychologic factors relating to food intake may interact at the diencephalic level, each exerting a modifying influence on the other.

Energy Expenditure. Energy balance is a matter of energy expenditure as well as of energy intake. Hence it is appropriate to mention briefly the role of activity in the genesis or prevention of obesity. Mayer[27] has shown that there is an enormous difference in spontaneous activity between hereditarily obese mice and non-obese control groups. The obese animals are practically inactive. However, this inactivity is not the result of extreme obesity but precedes it, as is shown by comparison of activity rates of non-obese animals and young pre-obese mice of the same weight.

Thus it is possible to ascribe to this decrease in activity a role in etiology of the obesity which develops somewhat later. Indeed, from a thermodynamic standpoint the rate of fat accumulation of mature hereditarily obese mice is inexplicable unless the role of activity is taken into account. When hereditarily obese mice carry the "waltzing" gene and are in constant

rotary movement in their cages, they rarely gain excessive weight.

It has always been assumed that in normal animals food intake bears a constant relationship to activity. Although this is true within the normal "physiologic" limits of activity, there are situations in which activity is so great that food intake lags behind and the subject concerned loses weight. A recent study has shown that, conversely, when activity is quite low, food intake tends to level off and will remain constant even while activity progressively decreases.[28] The result in this instance, of course, is weight gain. (Fig. 2.)

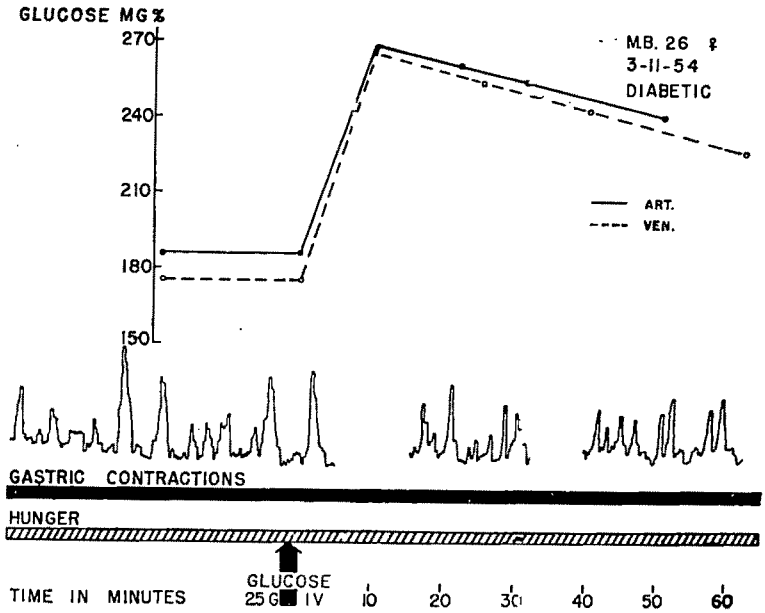


FIG. 1. Failure of intravenously administered glucose to abolish gastric hunger contractions in a patient with uncontrolled diabetes mellitus (Stunkard and Wolff[21]).

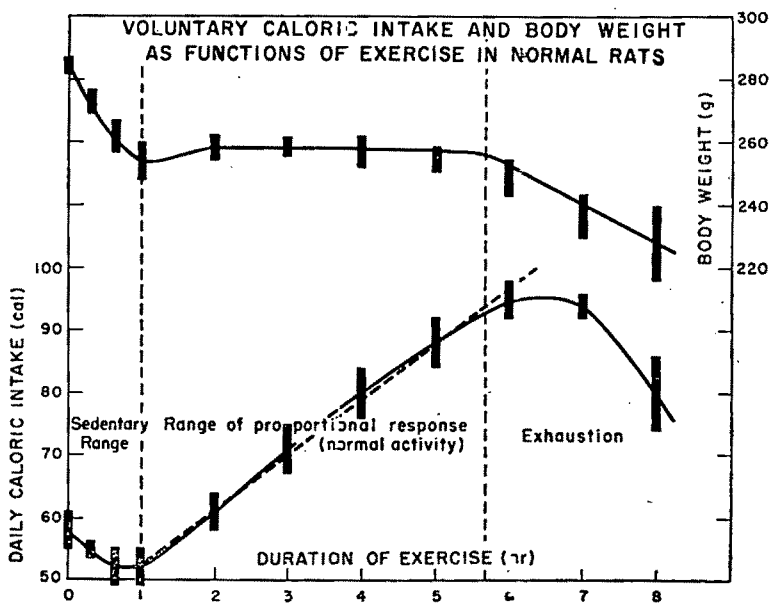


FIG. 2. Food intake and body weight as functions of duration of exercise in normal adult rats (Mayer et al.[28]).

These observations suggest that in man an increasingly sedentary existence may not be accompanied by a proportionate decrease in food intake. It is possible that the slowly progressing mild obesity of middle age may be explained on this basis.

Factors Influencing Fat Synthesis and Degradation. Metabolic obesity is considered to be primarily determined by the rate at which fat is formed or broken down. Of course, since injury to certain "regulatory" hypothalamic centers may affect metabolism, it is difficult to classify that form of experimental obesity in which injury to the hypothalamus is followed by a mixture of regulatory and metabolic abnormalities.[8–9]

Fat deposition and mobilization are under three forms of control acting synergistically, namely, enzymatic, hormonal and nervous. (It is worth being reminded that hormones do not initiate events within cells but merely regulate the rate at which some of the events occur.)

A major factor favoring fat mobilization and oxidation is lack of insulin. In diabetes mellitus and in the "diabetes" of carbohydrate starvation lipogenesis is greatly decreased and lipolysis is greatly accelerated. In order for glycolysis to continue, glucose must be able to enter cells. This it apparently cannot do readily without an effective supply of insulin. Moreover, the process of lipogenesis from 2-carbon fragments appears to depend upon glycolysis in that the energy needed for lipogenesis must be derived from the glycolytic process.[29]

Both in tissue slices and in intact animals it has been shown that the oxidation of longer chain fatty acids is strikingly influenced by carbohydrate. Geyer[30] has shown that fasting accelerates the oxidation of palmitic acid by rat liver slices. Pyruvate added to the medium decreases significantly the palmitate oxidation by such slices. In the intact rat oxidation of palmitic acid and tripalmitin is increased by prior fasting of the animal and decreased by prior glucose administration.[31]

Cellular carbohydrate deficiency also is associated with increased secretion of hormonal factors which are believed to stimulate fat mobilization. A pituitary substance may act to increase the rate of lipolysis; how this is accomplished is not clear.[32] The adrenal cortex also influences fat mobilization, apparently by playing a "permissive" role.[33] It has also been reported that epinephrine secretion and the integrity of the sympathetic nervous system are needed for effective mobilization of fat stores.[34–35]

When an adequate supply of insulin is available to the organism, lipogenesis is not inhibited and may increase if an "excess" of glucose is transferred into cells. Adipose tissue itself can synthesize fat. Shapiro and associates[36] have reported that adipose tissue *in vitro* will take up fatty acids from serum. Hausberger *et al.*[37] have compared the relative abilities of liver and of adipose tissue to form fat by measuring rates of conversion of radioactive glucose to fatty acids and carbon dioxide by both tissues. They demonstrated in normal animals that radioactive glucose is utilized for lipogenesis and is oxidized by liver and adipose tissue. By contrast, liver and adipose tissue of alloxan-diabetic rats are incapable of converting glucose carbon into fatty acids. Of particular interest is their finding that insulin administration *in vivo* restored hepatic and adipose tissue lipogenesis to normal levels and that continuation of insulin treatment promoted high levels of lipogenesis in both tissues. Administration of insulin to the alloxan-diabetic rats not only accelerated glucose metabolism to carbon dioxide and fat but also altered the channeling of energy so that much more fat was produced.

When we speak of adipose tissue as being more or less metabolically inert it is because we do not take into account the large mass of triglyceride usually stored in the depots. If the large difference between lipid and protein content in liver and adipose tissue is taken into account it can be demonstrated that adipose tissue is far more active than liver both in oxidizing glucose and in converting glucose to fat.[37] An excess of insulin has a more marked effect on lipogenesis in both tissues than upon glucose oxidation. Incidentally, the fact that more triglyceride is stored in the body in obesity does not mean that there is a larger amount of active adipose tissue. Hence the ability of the obese person to convert carbohydrate to fat should not be enhanced just because the quantity of fat stored in the adipose tissue is great.

According to Levin and Farber[33] adrenalectomy will greatly inhibit or prevent the fat mobilization which otherwise follows fasting, anterior pituitary extract administration or exposure to the appropriate stress. (Adrenalectomy also inhibits fat deposition.) If mice subjected to adrenalectomy are pre-treated with cortisone they can again mobilize their fat

in response to such trigger factors as APE, growth hormones and stress.

Hausberger[38] has shown that when the nerve connections supplying the symmetric interscapular fat body of the mouse are cut on one side only, an influx of glycogen and, in its wake, an accumulation of fat can be observed within ten hours after denervation. The fat content of the denervated side exceeds that of the control side in the normally fed as well as in the starved mouse. The denervated side can be depleted of fat only after long periods of hunger. As Wertheimer and Shapiro[39] point out in their excellent review, normal innervation seems a requisite for the attainment of a dynamic equilibrium in fat tissue.

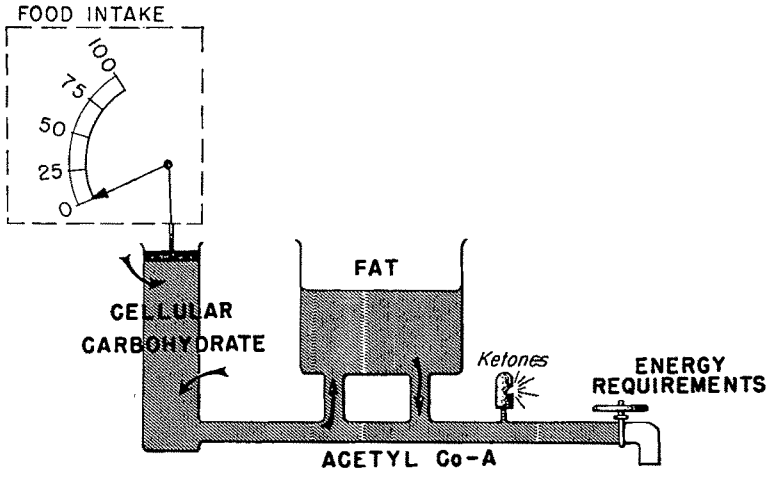


FIG. 3. Postulated relationship between energy requirements, cellular carbohydrate supply and food intake.

It appears, then, that the factors which favor lipogenesis are usually those which also favor entry of glucose into cells. Presumably, insulin acts to increase lipogenesis by accelerating transfer of glucose into cells and not by an effect within the cell. By contrast, factors which act to exclude glucose from cells favor increasing fat mobilization and oxidation.

Relation of Abnormalities in Fat Metabolism to Regulation of Energy Balance. In order to relate abnormalities in synthesis and metabolism of fat to an over-all imbalance of energy intake and energy expenditure, a mechanism capable of mediating between energy expenditure and food intake must be considered. Although such a regulator has not been delineated in a quantitative sense it appears that the concept of a "glucostatic" mechanism permits the gap to be bridged between an apparently primary disorder in fat metabolism and an associated disorder of energy balance. This postulated relationship is indicated in Figure 3.

Ordinarily carbohydrate and fat both serve as fuels for energy expenditure in a proportion presumably determined by the amount of carbohydrate available to peripheral cells. When, for example, the blood sugar falls to fasting levels or below, homeostatic mechanisms come into play which cut down drastically on the rate of peripheral glucose utilization, stimulate gluconeogenesis from protein, decrease lipogenesis and stimulate fat mobilization from the depots. Under such circumstances increasing amounts of fat are metabolized.

According to the glucostatic theory desire for food is also a part of the complex homeostatic activity designed to protect the organism, notably the central nervous system, against carbohydrate privation. In theory, if available carbohydrate were shunted too rapidly to fat or if the fat depots were unable to contribute their share of energy to metabolism, carbohydrate would be depleted at an abnormally rapid rate and food intake and fat stores would increase. In diabetic-like states food intake is frequently increased but, since lipogenesis is impaired under such circumstances, fat is lost rather than gained. Naturally, when energy requirements are increased, as in exercise or hyperthyroidism, spontaneous food intake usually increases proportionately. However, in such instances lipogenesis is curtailed somewhat and fat is readily utilized.

Adolph,[11] writing of the mechanism which

governs the urge to eat, states that "the investigator gains the feeling that some complex of internal compositions intermittently drives the animal to eat so that some resultant concentration is kept just above or below a threshold value." The supply of carbohydrate within the cell seems to correspond well to Adolph's postulated "complex of internal compositions." The supply of cellular carbohydrate depends upon the availability of blood glucose, and blood glucose is readily available to non-nervous cells only during periods of relative hyperglycemia. Such intervals of hyperglycemia, in turn, ordinarily occur only after meals. A relationship between the rate carbohydrate is utilized and the energy requirements of the body has not been worked out. However, the rate of carbohydrate utilization seems to correspond better to the rate of energy expenditure than does the degree of S.D.A. obtained after a meal. Such a glucostatic theory explains better than the thermostatic theory the hyperphagia associated with uncontrolled diabetes mellitus, hyperthyroidism and treatment with corticotropin and cortisone.[6] Finally, since maintenance of a certain minimal glucose level is absolutely indispensable to life, the body would be expected to ensure regular replenishment of its supply of carbohydrate from outside sources.

TABLE II

"CLINICAL" COMPARISON OF THREE FORMS OF EXPERIMENTAL OBESITY

Variety of Obesity	Hereditary Obesity-Diabetes Syndrome (mouse)	Gold-Thioglucose-Induced Obesity (mouse)	Hypothalamic Obesity (rat)
Origin	Recessive mendelian entity	Injection of LD_{50} of gold thioglucose	Electrolytic lesions of ventromedial nuclei
Adjustment to dietary dilution	Relatively normal	Abnormal	Abnormal
Spontaneous activity	Markedly diminished	Normal	?Normal
Oxygen consumption	In proportion to lean body mass, not body surface	Follows surface law (splanchnomegaly occurs with obesity)	Normal
Resistance to cold	Poor	Normal	Normal
Response to insulin	Insulin resistant	Normal	Sometimes insulin sensitive
Response to growth hormone	Marked hyperglycemia in response to growth hormone injection	Normal	Normal
Blood glucose level (non-fasted)	Hyperglycemic after 8th week	Normal	Lower than in controls
Endocrine anatomy	Increase in number and size of pancreatic islets	Normal	Gonadal atrophy, renal lesions
Response to high fat diet	Loses weight	Obesity accelerated	Obesity accelerated

If the normal relationship between energy requirements and cellular carbohydrate supply were disturbed by an appropriate metabolic disorder, it should be possible according to the glucostatic hypothesis, to predict that obesity will occur. A consideration of certain recent metabolic studies made on obese animals may serve to illustrate the prediction value of this hypothesis.

Experimental Obesity. Three forms of experimental obesity have been carefully studied, hypothalamic obesity,[6,17] gold thioglucose obesity[40–41] and the hereditary obesity-hyperglycemia syndrome in mice.[42–43] These forms of experimental obesity cannot be discussed in detail in this presentation but certain of their clinical characteristics are compared in Table II.

It is of particular interest, however, to determine whether or not these forms of obesity can be understood in terms of some disordered mechanism or mechanisms. One of the first questions that might be asked about these three forms of obesity is whether they differ in terms of body composition. Bates and coworkers[44] have shown that the iodine and saponification numbers of the triglycerides synthesized by the three groups of animals are similar to those of their non-obese controls. The total cholesterol content of the hereditarily obese animals is higher than it is in their controls. All of the obese animals display an increase in liver weight and, of course, in total fat content.

When incorporation of labeled acetate into fatty acids is determined in the three forms of experimental obesity the metabolic differences between them are more clearly defined.[45] For example, hereditarily obese mice with slight caloric deficit retain much more dietary acetate as carcass fatty acids than do their sibling controls. When hypothalamic hyperphagic rats and genetically obese mice are fed one-half the number of calories previously found necessary to maintain caloric equilibrium, they retain significantly more C^{14} from dietary radioacetate in carcass fat than do their controls. Gold thioglucose obese mice in negative caloric balance retain the same amount of C^{14} from labeled acetate as do their controls. Since the specific activity in the fat of the obese animals is the same as that in their controls, the increased retention of the label observed in the hyperphagic rats and genetically obese mice cannot be regarded as a dilution phenomenon.

The fact that there is a relatively increased retention of C^{14} from radioacetate during negative caloric balance in the hypothalamic rat suggests that the operation has produced a profound alteration in the metabolic pattern. It is also of unusual interest that the genetically obese mice continue to synthesize fat at an appreciable rate even during caloric restriction, a circumstance associated with drastic diminution of lipogenesis from acetate in normal animals. Such findings are consistent with the observations of Alonso and Maren[5] that genetically obese mice underfed for six months, so that their body weight was 30 per cent less than that of their non-obese siblings, still contained several times as much fat as their controls.

Bates, Mayer and Nauss[46] also have studied fatty acid turnover in the three forms of obesity under consideration. Radioactive carboxyl labeled palmitic acid was used as the tracer and the average amount of fat mobilized during the experimental period was calculated by measuring the decline in isotope content of carcass fat with time. If the means of the amount of fat mobilized daily are assumed to be fairly accurate, it is possible to calculate the per cent of fat gain that is due to decreased mobilization and that due to increased synthesis (Table III). Such calculations suggest that the fat accumulated by the gold thioglucose-obese animals can be accounted for to some extent by decreased mobilization, while the fat accumulated by the obese-hyperglycemic mice appears to result about equally from decreased mobilization and increased synthesis. The hypothalamic rats show a remarkable decrease in fat mobilization.

TABLE III
CALCULATED ORIGIN OF INCREASED FAT CONTENT IN THREE FORMS OF EXPERIMENTAL OBESITY*

Rates	O-H Obese Mice	G.T.G. Obese Mice	Hypothalamic Rats
Expected rate of fat Accumulation per day (g)	.13	.18	1.12
Actual rate (g)	.27	.18	1.76
Synthesis rate (per cent of control)	152	100	154
Mobilization rate (per cent of control)	56	60	5

* Bates, Mayer and Nauss.[46]

It is worth pointing out that one of the strong arguments against the "lipophilia" explanation of obesity has been the fact that adipose tissue cells of obese persons do not appear to resist mobilization of fat during undernutrition. A number of nitrogen balance studies have indicated that obese persons utilize their own fat as readily as do non-obese individuals.[2] For this reason it would be premature to interpret human obesity in terms of the metabolic behavior of experimentally obese animals. Nevertheless, these preliminary observations on fat metabolism in three forms of experimental obesity appear to illustrate several of the postulated metabolic disorders which it was predicted could result in obesity.

To summarize, an attempt has been made to view obesity as an end product for which a number of disorders, each affecting energy balance in its own way, might be responsible. A tentative classification of "obesities" in terms of etiologic mechanisms is proposed. Such disorders appear to fall into two broad categories, those affecting primarily the regulation of energy balance (regulatory obesity), and those affecting primarily the intermediary metabolism of foodstuffs and fat turnover, and secondarily energy balance (metabolic obesity).

It is believed that the "glucostatic" theory of food intake regulation is of considerable heuristic value in approaching the vast, complex problem of human and experimental obesity. Although this theory cannot be regarded as established at the present time, it appears to account more satisfactorily than other contending formulations for the cyclic nature of hunger and satiety on the one hand and the conditioning effect of energy requirements upon the urge to eat on the other.

Lastly, metabolic studies on fat metabolism in three forms of experimental obesity have been described in support of the view that the etiology of obesity can be multiple and that the unitary concept of obesity in man as a simple matter of overeating requires critical re-examination.

DR. TAGGART: We should like to turn our attention now to certain of the basic principles in the clinical management of obesity. I know of no more challenging problem for the practitioner of medicine. Our writer, Cyril Connolly, has said that, "Imprisoned in every fat man a thin one is wildly signalling to be let out." Unfortunately, when the obese patient is confronted with a suitable regimen for weight reduction, the thin man inside too often begins to signal less and less frantically. I hope that Dr. Sidney Werner will tell us, if he can, how this problem can be managed least painfully.

DR. SIDNEY C. WERNER: The success of the long-term treatment of obesity is apt to be slight. Nevertheless, the results often are enough to encourage further efforts. Any treatment must follow the usual principles either of trying to attack the cause of the disorder or of rectifying, if possible, some resulting physiologic abnormality. This discussion will not include those situations in which an evident physiologic disorder is the cause of obesity, such as hyperadrenocorticism, adenoma of the pancreas and hypothalamic injuries.

Some years ago Dr. Hilde Bruch, at the Babies Hospital in New York, became interested in the problem of the etiology of obesity and was especially struck by the parental relationship that existed between the mother and the obese child which, she concluded, led to the child's overeating. In short, the increase in appetite was based not upon a need for food but upon the fact that the child responded to emotional upset by eating. In the majority of obese people observed at the clinic it was believed that this emotional origin was more often the cause of their overeating than was any physical defect. There are many pathways by which emotional overflow can result in an increased appetite.

Dietary restriction, that is decreasing caloric intake in relation to caloric output, has been the fundamental principle behind therapy for obesity. The work of Dr. Newburgh many years ago showed clearly that reduction in caloric intake below the body's needs inevitably leads to weight loss. Unfortunately, upon the institution of starvation (which is what such caloric reduction really means) there is a corresponding reduction in the rate of basal metabolism. With reduction to levels as low as −30 per cent, what was originally a low calorie diet may soon become a maintenance one.

It is also well to bear in mind that certain small individuals have basal metabolism requirements which are not much greater than 1,200 or 1,400 calories, so that 1,200 calories may be far from a low calorie diet. Most people like to feel satisfied after having eaten. A low caloric diet does not have a large bulk so that those placed on such a regimen are likely to feel disturbed and hungry.

Attempts have been made to tackle the problem of weight reduction by increasing heat loss. High protein diets have been used for this purpose on the assumption that specific dynamic action can be increased and hence the patient can be made to expend more energy. It can be said from personal experience with several patients in the metabolism ward that the amount of weight loss produced by a 200 gm. protein diet is insignificant, and I do not feel that this regimen is a very successful one.

The use of a high fat diet has also been recommended by Pennington. The theory was partly suggested to him by the work of Mayer and his associates, and held as its essential principle that fat introduced into the metabolic mixture will

be burned without being involved in the metabolism of glucose. This hypothesis is open to criticism since it is almost certain that excess of fat can cause fat deposition without the need of fat entering the Krebs cycle. However, the practical fact is that when one administers a high fat diet, some patients lose weight. This has been borne out in our experience and, of course, one wonders why. Have the laws of thermodynamics been disproved?

If fat was burned and not utilized for anabolism, a large heat output from the body with a high fat diet could explain how a patient would fail to gain or would actually lose weight. However, the work of Newburgh many years ago showed clearly that, although obese people do have an increased heat output, it is not out of proportion to their increased body mass. Thus there is no evidence that obese people eliminate excess heat indicating excessive catabolism of fat.

In order to pursue further the question of why some people lose on a high fat diet containing as much as 2,800 calories, patients were admitted to the metabolism ward for study. I will show the results and some of the difficulties in such a study.

The first patient showed an abrupt loss in weight when placed on the diet originated by Dr. Pennington. He then lost less weight on an isocaloric high carbohydrate diet and had another drop in weight on a second trial of the Pennington regimen. Unfortunately, however, this patient developed diarrhea on the high fat diet.

The second patient maintained weight with the high fat diet, then increased her weight on the standard diet. However, it is well known that in the shift from a high fat to a high carbohydrate diet there is retention of salt and water. Thus it became evident that fluid, sodium and potassium balances had to be determined in order to give meaning to the experiment.

This was done with a third patient who showed a slight decline in weight under the influence of the Pennington regimen. There was sufficient shift in her sodium and water balance to make it seem likely that that was all that was happening.

Similarly, a fourth patient developed slight weight loss on the high fat regimen, accounted for largely as loss of salt and water. I should mention that the last two patients, who had been amenorrheic for at least six months or a year, started menstruating two days after the baseline diet period was completed and the Pennington diet begun. As you know, salt and water are retained just before menstruation and are subsequently lost.

The last two patients show clearly what happens with a 2,800 calorie high fat, low carbohydrate diet and with a diet of isocaloric content high in carbohydrate and low in fat. In these two men there was virtually no difference in the loss of weight between the two diets.

To discuss a different type of approach, the use of a low salt diet in dietotherapy has been recommended. Essentially, I think, its value lies only in decreasing the palatability of the diet.

Still another approach has been the effort to influence appetite adversely. The analeptic agents, such as benzedrine® and dexedrine,® have been used. It is probable that these agents are effective but they soon lose their efficacy and, at a time of emotional upset, they are little protection against the urge to eat.

Just as glucose given intravenously decreases appetite, amino acids, particularly glutamic acid, given intravenously have a very potent anorexigenic action. For that reason a series of products containing amino acids for oral use have appeared on the market. We were asked to appraise one of these products and set up an experiment in which there were three regimens: (1) the particular preparation with added amino acid; (2) a placebo; and (3) adhering only to the instructions accompanying the preparation advising the patient to limit diet, eat lettuce, bulky foods, and so on. It was interesting to see that by following the last regimen the patients lost somewhat more than with either the placebo or the amino acid preparation in question.

Finally, several preparations have been introduced which are designed to dull the sense of taste. As Dr. Van Itallie has just pointed out, these are not very rewarding.

Attempts to provide psychotherapy, when an emotional derangement is the cause of the obesity, suffer from several handicaps. For one, there appears to be an age limit beyond which psychotherapy has little value. In fact Dr. Richardson at the New York Hospital made a careful psychoanalytic survey of several patients and concluded that after a certain age the protection provided by the obesity is so necessary to the individual that it is dangerous to attempt psychotherapy or too vigorous weight reduction.

At any rate, intensive psychotherapy is not economically feasible for most people. However, mild psychotherapy through the patient's own doctor is extremely valuable and is readily available. The fact that a doctor sees an obese patient and doesn't look repelled or disappointed is often in itself psychotherapeutic. These obese people who are somewhat dependent often do better in the hands of a sympathetic physician than when consulting one who has no interest in the problem.

There are several drugs designed to stimulate the basal metabolic rate which should be mentioned. Dinitrophenol we all know to be so toxic that its use can never be recommended. As for the use of thyroid gland preparations, many studies have shown that unless toxic doses are used the patient's own thyroid gland has a remarkable capacity to decrease its function in proportion to the amount of thyroid administered. Thus very little is accomplished except for a week or two.

Exercise is of little avail in weight reduction since the human body can do considerable work on a small amount of food. To reduce weight by exercise in the way Dr. Van Itallie's rats did leads ultimately, as it did in them, to exhaustion. Moreover, many people after exercise eat more than enough to make up for the calories expended in this way.

Massage is even more futile and there is no evidence that increasing local circulation in this way mobilizes fat depots.

DR. TAGGART: Thank you, Dr. Werner. That completes the formal part of the program. We have allowed time for discussion, and I am happy to see that we have with us this morning Dr. Hilde Bruch and Dr. Albert J. Stunkard.

There was one point in therapy which was not mentioned and concerning which I think Dr. Van Itallie has some information, that is, the use of hydrophilic colloids.

DR. VAN ITALLIE: We have recently completed a clinical study involving comparison of the anorexigenic effects of a hydrophilic colloid preparation and a preparation containing amphetamine. Approximately sixty obese patients who are being observed in our Nutrition Clinic served as subjects. Alternate patients received tablets identical in appearance to be taken three times per day before meals. One tablet contained 200 mg. of carboxymethylcellulose, the other 200 mg. of carboxymethylcellulose plus 10 mg. of amphetamine. In all other respects the patients were treated in the same way.

For the first six weeks the patients given the preparation containing amphetamine lost an average of 0.77 lb./week. For the entire thirty-week period of study the same group (which diminished in number as well as in weight as time passed) lost an average of 0.55 lb./week. The patients on colloid alone lost an average of 0.15 lb./week for the first six weeks. For the entire thirty weeks their weight loss averaged 0.10 lb./week. The differences between the means of the two groups were statistically significant. Moreover, when another group of patients was switched from the colloid to the tablet containing amphetamine without their knowledge a statistically significant increase in weight loss followed. Another control series of thirty-five patients who had been seen in the clinic on another occasion and who had received no medication lost slightly less weight on the average than the group treated with amphetamine (0.43 lb./week over a thirty-six week period).

These results indicate that the colloid preparation in the quantities prescribed was of no value and may have interfered with the reducing regimen by providing the patient with something he thought was a crutch. By contrast the amphetamine seemed to have a definite anorexigenic effect, particularly during the first six weeks of therapy. However, the results obtained in the second control group receiving no medication at all suggest that amphetamine is of limited value in the management of obesity. It is no substitute for sympathetic supervision by the physician but it may be temporarily helpful if used in conjunction with such supervision.

MR. FRED KATZ: Why does basal metabolism go down during fasting?

DR. WERNER: That is an interesting question. Efforts to study thyroid function under conditions of starvation suggest that the thyroid remains more or less intact. However, it is quite interesting that corticosterone in thyroidectomized patients will increase the metabolic rate quite sharply. There is a recent paper about a growth hormone describing a non-specific increase in the metabolic rate not mediated through the thyroid. If one developed an adaptation to starvation through the adrenal or the pituitary, one could quite readily have an extrathyroid decrease in metabolic rate.

MR. BYRON HARDIN: Is there any evidence

that thyroid substance could overcome the effect of that decreased rate?

DR. WERNER: Well, not so far.

MR. PETER ROWLEY: Querido claimed that in two groups of rats on isocaloric diets, one group getting all their food at one time and the other having it spread throughout the day, different rates of fat accumulation resulted. The group receiving the single massive meal showed a definite gain of weight over those who received the food over the course of the day. Is that true?

DR. WERNER: That is quite true. Perhaps it is a matter of specific dynamic action. There is evidence to indicate that the flare-up three times daily in specific dynamic action is greater than that with the same amount of food given in a single meal. Is that your impression, Dr. Van Itallie?

DR. VAN ITALLIE: I think that is partly the explanation. Brobeck and Tepperman were able to demonstrate a similar rapid rate of fat accumulation when they were trying to explain the remarkably high respiratory quotients exhibited by hypothalamic animals after ingestion of food. They fed normal rats a full day's ration within a two-hour period and observed that animals trained to eat in this manner developed an extraordinary ability to convert ingested carbohydrate and, presumably, protein to fat. I assume that these particular animals converted an equivalent quantity of fat into energy later in the day. However, had they been allowed to eat another meal some hours hence, obesity might well have ensued.

DR. TAGGART: Quantitatively, how important a factor is specific dynamic action?

DR. WERNER: I think it is about 6 to 10 per cent of the energy requirement.

DR. VAN ITALLIE: Protein alone is about 10 per cent. The lower figure occurs with a mixed diet.

DR. TAGGART: I was surprised to hear the thermostatic theory revived. Is this not really another version of the old "luxuskonsumption" advocated many years ago, namely, that heat production could increase, *pari passu*, with caloric intake? I thought Newburgh answered that when he told of putting an individual on a 3,500 calorie diet with a resultant heat production of some 2,900 calories per day. When the caloric intake was increased to 5,000 calories a day, heat production went from 2,900 to about 3,000 calories.

DR. R. J. CROSS: May I ask about the glucostatic theory? It was suggested that the level of glucose within the cells might be responsible for appetite. I have wondered if it might not be some breakdown product of glucose rather than glucose itself, pyruvate, for example, or something of that sort.

DR. VAN ITALLIE: The only evidence I know of is the previously cited work of Larsson and Forssberge. You will recall that they demonstrated a preferential uptake of radiophosphate by hypothalamic tissue in the area of the "feeding" center when the rats under study fasted. We know that phosphate enters cells with glucose in many instances; therefore, the supply of phosphate to the cell might be crucial. I think that whatever the crucial reaction is, whether it involves some breakdown product of glucose, high energy phosphate bonds, intracellular potassium or something else, the proponents of the glucostatic theory tie it to the carbohydrate supply. Thus the availability of carbohydrate to the postulated glucoreceptor is seen as the limiting factor regardless of the events which take place after the glucose has entered the receptor cell. All this, of course, is highly theoretical and speculative.

DR. TAGGART: Dr. Bruch, I would like to ask you a loaded question. We have devoted most of our time today to a consideration of physiologic and biochemical factors which may operate in the deposition and mobilization of fat. Would you care to tell us how important you believe these factors to be in comparison with the psychological factors which operate in a great many cases?

DR. HILDE BRUCH: You certainly are loading the question by putting it as an either/or proposition. I think the psychological factors are terribly important. Their historical development is of interest. In my first studies on obesity I reviewed the literature and ran across all the different ideas. I felt that they made sense for a psychosomatic approach but that we did not have sufficient data on the metabolic aspects. Therefore, my answer to your question is that the basic physiologic facts are so important that I can think of no better way of progressing in this problem.

But these discoveries have nothing to do with the answer to the second part of your question. The psychological problems are also important. It is fairer in our approach to patients not to deal with these problems as mutually exclusive.

Dr. Van Itallie separated them as two different forms in his classification. According to my way of thinking, they are interrelated and are not two separate problems.

The best part of the new work will be its application to the management of obesity. This new knowledge lifts the approach out of the realm of the ridiculing, the moralizing and the condemning that has so often characterized the attitude toward fat people. It permits a more positive approach to these patients so that they will be treated with the respect they deserve.

DR. ROBERT F. LOEB: Is it fair to say, regardless of what group of factors play what relative roles, that we still have to reduce the number of calories (1) below the patient's appetite and (2) to a point where he actually loses weight? From the point of view of the physician, I think the most important thing in the management of these patients is to let them know that we know it is as difficult for them to reduce their caloric intake to the point of negative nitrogen balance as it would be for the rest of us who are not obese to go on a starvation regimen month in and month out, year in and year out. If this is brought to the attention of the patient I think we are doing a great deal toward helping him with his own problem. He must be helped.

DR. BRUCH: I would go one step further and raise the fundamental question of whether it is really good medicine to approach the weight problem directly. Again, I refer to follow-up studies. I have seen many patients who, if the weight they had lost over twenty years was added up, should have disappeared; yet there they are very much in existence. All this effort, self-restraint and self-denial are completely wasted. It seems that many fat young people go back to their previous weight (or a bit higher) after efforts at reducing. This is in agreement with the observations that have been made on starvation. If after a starvation experiment the body has gained complete restitution, the drive for excess eating persists for some time. It seems to me that Dr. Loeb's approach is the correct one. One should let the patient know that he will react to starvation like any normal person. This is one clue to the puzzle that many fat people, after an unjustified reducing regimen, will become even heavier. They react exactly like normal people after starvation. They continue overeating.

DR. ALBERT J. STUNKARD: There is another question I would like to ask in regard to the selection of patients who are dieting. An occasional patient is observed who, if placed on a diet, becomes seriously disturbed or psychotic. I wonder if Dr. Bruch might make some remarks in regard to the selection of patients?

DR. BRUCH: That is the point Dr. Werner mentioned, that Dr. Richardson had made certain observations on the development of neurotic symptoms in fat people. Dr. Richardson studied patients who were somewhat advanced in years. My observations have been made chiefly on adolescents, and it seems to me that they as a group are even more fragile in their total adjustment. When one approaches their problem by forcing them to lose weight, they are apt to develop serious mental symptoms. The fact that they are fat suggests that their life adjustment was poor at the start. They have been persecuted for being too heavy and feel rejected as people. The weight becomes an issue in a precarious mental balance. Enforced reducing is an attack on their security and may lead to psychotic reaction, that is, to a frank schizophrenic episode. In this group of patients I have also seen benzedrine addiction or, more correctly, something that has a delusional character. It is unlike addiction in which a patient feels physiologically tied to a drug. The delusional aspect is the conviction that "without these pills, I cannot function any more."

SUMMARY

DR. GILBERT H. MUDGE: Obesity is a condition which constitutes a serious public health problem because of the frequency and severity of its associated complications. In today's clinic, obesity has been considered in terms of physiological and biochemical mechanisms as well as in terms of the therapeutic procedures available to the clinician.

The physiological mechanisms may be divided into those primarily associated with total energy balance of the organism and those primarily related to disturbances of intermediary metabolism. The problem of food intake has been discussed in terms of the control of appetite by discrete functions of the central nervous system. The prevailing theories of the physiological basis of satiety have been reviewed. Hypothalamic centers, which are probably sensitive to glucose or other related metabolites, have been described. The function of these

centers has been analyzed in terms of the glucostatic theory of appetite control.

The metabolism of fat is regulated by enzymatic, hormonal and neural factors, and these are briefly reviewed. Adipose tissue itself has been found to have a surprisingly active metabolism. Three types of experimental obesity are discussed in terms of disturbances of specific metabolic reactions. These demonstrate the possibility that, from a metabolic point of view, obesity may be considered in terms of multiple etiologic factors.

In considering the various forms of therapy it is concluded that dietary restriction of caloric intake must still be considered the major component of any therapeutic program for obese persons. Psychiatric factors are frequently found to be responsible for the discrepancy between appetite and energy expenditure which results in clinical obesity. It is therefore emphasized that the advisability of weight reduction must be carefully evaluated in relation to the emotional problems presented by each individual patient.

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Clinico-pathologic Conference

Right-sided Heart Failure Occurring during Successive Pregnancies, Polycythemia and Sudden Peripheral Vascular Collapse

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THIS twenty-seven year old white housewife (No. 237929) was admitted to the Barnes Hospital on two occasions in the four months prior to her death. Her first admission was on May 27, 1954.

The patient had been in good health until twenty-four months before admission when she noted mild dyspnea on exertion during the third month of her second pregnancy. Shortly thereafter her ankles became swollen to a mild degree. These symptoms did not progress, however, and she had no further difficulty before or after delivery. Six months later she developed the same symptoms of dyspnea and ankle edema during the fourth month of her third pregnancy. Subsequently she became easily fatigued, gained weight slowly, and was delivered of a premature infant after seven months' gestation.

Her general health improved slightly after delivery until six months before the next admission, when she consulted her physician because of a recurrence of ankle edema. She was told that she had a "goiter" and an "enlarged heart." She was treated with digitalis and Lugol's solution, 15 drops per day, which resulted in moderate improvement. Four months before admission she discontinued the medications without consulting her physician. One month later she began to experience attacks of palpitation due to the sudden onset of rapid heart action followed by an abrupt disappearance of this symptom. Three months before admission her legs became progressively larger, eventually becoming swollen above the knees. During this time she was moderately dyspneic but not orthopneic. Two weeks before admission her abdomen became markedly swollen, and she again consulted her physician. At this time she was redigitalized, placed on a low salt diet and given intravenous injections which resulted in rapid loss of the edema. A week before admission she noticed that her fingernails had become a dark blue, and she complained of a "tight feeling" in her chest. She was immediately hospitalized and given oxygen which resulted in subsequent clearing of the cyanosis. While in the hospital she was again given Lugol's solution, 10 drops per day. When after five days she failed to improve she was transferred to the Barnes Hospital.

The past history revealed that the patient had been the product of a normal delivery. There had been no cyanosis at birth, no known congenital defects, nor any unusual or severe disease during childhood and adolescence. Seven years before admission she had consulted a physician because of dysmenorrhea which was ascribed to a "tilted womb." At that time she was told that she had an "enlarged heart" and "palpitation of the heart," although she had not noticed any cardiorespiratory symptoms. The condition of the patient's mother, age sixty-three, had recently been diagnosed as "rheumatic heart disease." One sister had had a "toxic goiter" removed at Barnes Hospital.

The personal and social history were not contributory.

Physical examination at the time of admission revealed the patient's temperature to be 37.6°C.; respirations, 16; pulse, 120, and blood pressure 98/68. She weighed 84 pounds. The patient was a well developed, small, chronically

ill white woman in no acute distress. The skin was warm and slightly moist and there was no evidence of cyanosis. There was no significant lymphadenopathy. An acute chalazion of the right upper eyelid with erythema, swelling and tenderness, especially of the lateral portion, was noted. No proptosis of the eye or drooping of the lid was encountered. The optic fundi were normal. Further examination of the head and neck revealed only an asymmetrical goiter with the left lobe and isthmus enlarged to twice normal size. The lungs were not remarkable. Examination of the heart revealed a point of maximum impulse in the fifth intercostal space at the mid-clavicular line. On percussion the left border of cardiac dullness was encountered at the anterior axillary line. The cardiac rate was rapid; the rhythm was regular. A proto-diastolic gallop rhythm was heard over the cardiac apex. A grade I, low pitched murmur was heard in middle and late diastole over the cardiac apex in the anterior axillary line, and a soft grade I systolic murmur was noted along the left sternal border. The first heart sound at the cardiac apex was not accentuated. The second heart sound over the pulmonic area was increased in intensity. Examination of the abdomen revealed no abnormalities except for a firm liver edge palpable two fingerbreadths below the right costal margin. Only a trace of ankle edema was present. Results of the neurologic examination were within normal limits.

The laboratory data were as follows: hemoglobin, 20 gm. per cent; red blood cells, 6,500,000; white blood cells, 8,500; differential, normal. Urinalysis, specific gravity, 1.017; pH 5.0; protein, 1 plus; sugar, negative results; centrifuged sediment: 8 to 10 white blood cells and 1 to 3 red blood cells per high power field. Stool guaiac test gave negative results. Blood cardiolipin, negative results. Blood chemistry: non-protein nitrogen, 27 mg. per cent; total proteins, 7.0 gm. per cent; albumin, 4.0 gm. per cent; sodium, 145.9 mEq./L.; potassium, 4.7 mEq./L.; carbon dioxide combining power, 24.2 mM/L.; protein-bound iodine, 11.4 μg. per cent. Electrocardiogram: right ventricular enlargement; sinus tachycardia; auricular enlargement; digitalis effect. Roentgenogram of the chest: cardiac enlargement, left auricular and right ventricular; pectus deformity. Venous pressure, 240 mm. of saline. Antistreptolysin "0" titre, 250 units. C-reactive protein, 3 plus. Throat culture: moderate growth alpha streptococci; few pneumococci. Blood culture, no growth. Basal metabolic rate, −3 per cent.

The patient's course in the hospital was uneventful. A therapeutic regimen was maintained consisting of a low salt diet and digitalis leaf in maintenance dosage by mouth. She remained afebrile and free of respiratory distress. The chalazion of the right eye responded promptly to the local application of bacitracin ointment. During hospitalization many cardiopulmonary diagnoses were suggested. On June 7, 1954 the patient was subjected to cardiac catheterization. The results were as follows:

Source	Blood Oxygen Content (vol. %)	Blood Oxygen Saturation (%)	Pressure (mm. Hg)
Pulmonary artery	11.96	57.3	80/45
Right ventricle (high)	12.77	61.2	80/0
Right ventricle (low)	11.82	56.8	
Right auricle	11.84	56.9	
Superior vena cava	10.99	52.5	

Pulmonary capillary pressure was 9 mm. of mercury. Simultaneous arterial oxygen saturations from the brachial and femoral arteries each yielded values of 98 per cent. The results of these studies were interpreted as follows: "Mitral stenosis is very unlikely. There is pulmonary hypertension. There is no evidence of a left to right shunt." The patient was discharged on June 15, 1954.

On September 16, 1954 the patient was brought to the emergency room in extremis. She had been seen a week before in the cardiac clinic and seemed to be doing well. Her polycythemia was again noted, and plans had been made to rehospitalize her soon to study the cause of the increased red cell production. The day before admission it was noted that her face and neck had begun to swell. Shortly thereafter she became nauseated, complained of palpitation, vomited, and retched frequently. She became very apprehensive and quite restless. She was taken to her local physician who advised that she be brought immediately to this hospital.

Physical examination on admission revealed her temperature to be 37.0°C., respirations, 30, and pulse, 130. The blood pressure reading was unobtainable. The patient appeared very ill and was obtunded. The skin was ashen gray in color,

cool and moist. The lips were cyanotic. The head and neck revealed no change from the previous examination except for the neck veins, which were distended. The lungs were not remarkable. Examination of the heart revealed a forceful point of maximum impulse just to the left of the mid-clavicular line. The cardiac rhythm was regular, and a protodiastolic gallop rhythm was heard at the cardiac apex. No peripheral arterial pulsations were palpated except over the femoral arteries bilaterally. The edge of the liver was palpable four finger-breadths below the right costal margin. A mild degree of pitting edema of the thighs and sacrum was present. Neurologic examination was not remarkable.

The laboratory data were as follows: hemoglobin, 18.5 gm. per cent; white blood cells, 13,950; differential: stabs 6 per cent; segmented forms, 83 per cent; lymphocytes, 8 per cent; monocytes, 3 per cent. The electrocardiogram revealed no change from the previous record.

Within two hours after arrival in the emergency room the patient expired. During that period she remained dyspneic, restless, cyanotic, and in a state of vascular collapse.

CLINICAL DISCUSSION

DR. HARRY L. ALEXANDER: This case today is one that excited a good deal of interest on the wards. The patient was a young woman of twenty-seven. Past history was non-contributory. She began to suffer from signs of cardiac decompensation during her second pregnancy twenty-four months before her death. She recovered from this with the birth of her child. The third pregnancy came some six months after the second and was again characterized by cardiac failure. After the delivery of her premature child she recovered partially but the last six months of her life were made miserable by progressive cardiac failure. The one noteworthy aspect of her history is that at the age of twenty, during an incidental illness, she was told that her heart was large, although she had no symptoms. Furthermore, in the last year or two of her life a goiter was discovered. Presumably it was a toxic goiter for which she was given Lugol's solution. On her first admission the physical signs, aside from the goiter, were entirely referable to her cardiac embarrassment. She exhibited a high venous pressure, accentuated pulmonary second sound, an enlarged right ventricle by x-ray, and electrocardiographic evidence of enlargement of the right auricle. She was not cyanotic, which is an important observation. The only significant laboratory observation was a considerably increased red blood cell count. She became compensated during this first admission and did well for the three months after she returned home. She then came in again and died rather suddenly. Now may we see the x-ray films?

DR. WILLIAM B. SEAMAN: Conventional x-rays of the chest were taken on the day following hospital admission. A deformity of the thoracic cage and a depression of the sternum, the so-called pectus deformity, were noted, which is important to consider in evaluating the cardiac size because the thoracic cage deformity does tend to displace and rotate the heart, complicating the problem of chamber analysis when cardiac enlargement exists. The heart was obviously enlarged, the apex extending to the sixth anterior interspace a few centimeters lateral to the left mid-clavicular line. Usually this is considered to be evidence of left ventricular enlargement; however, there was definite right ventricular enlargement. In the presence of enlargement of the right ventricle it is difficult to determine radiographically whether there is left ventricular enlargement as well. Films made during barium swallow did not present very good evidence for left auricular enlargement. Prominence in the region of the main trunk of the pulmonary artery was also noted, suggesting that this artery may have been a little dilated. The lung fields were of particular interest in this case since the vessels were certainly normal in size, perhaps even a little less than normal. We had no radiographic evidence of pulmonary hypertension, even though it was present clinically. It is true that we have had cases of true pulmonary hypertension in which the vessels were not large; the explanation for this I do not know. There was also a film taken at the time of cardiac catheterization which suggested a tremendous enlargement of the right ventricular cavity, thus tending to confirm our impression of the conventional films.

DR. ALEXANDER: There are two points, Dr. Seaman, that puzzled us. It was very difficult, without being somewhat extravagant with our imagination, to understand why the left auricle was enlarged. Do you think that we may forget about that?

DR. SEAMAN: I personally do not feel that it was enlarged.

DR. ALEXANDER: Secondly, we were puzzled over the fact that the pulmonary conus was not appreciably enlarged.

DR. SEAMAN: Most of the films suggest that there was pulmonary artery dilatation. On the other hand, a very similar bulge in that area can result from right ventricular enlargement alone.

DR. ALEXANDER: Did the infundibulum of the conus appear enlarged?

DR. SEAMAN: We couldn't see that because it was the portion just beneath the valve.

DR. ALEXANDER: On the basis of the clinical findings, the suggested diagnoses were mitral stenosis or some form of congenital heart disease, more likely Eisenmenger's complex. Would you comment on these, Dr. Massie?

DR. EDWARD MASSIE: The first diagnosis that would have to be entertained clinically on this patient would be mitral stenosis if the auscultatory findings are correctly listed in the protocol. This would comprise the diastolic murmur at the apex, the accentuated pulmonic second sound and the enlarged right ventricle. Those add up to mitral stenosis, but wasn't some murmur heard at the left sternal border or at the pulmonary area? Were the murmurs in this patient heard only at the apex?

DR. ALEXANDER: Everyone who observed the patient heard a murmur along the left sternal border that was systolic in time, but not diastolic.

DR. MASSIE: We do know that in a septal defect, either auricular or ventricular, we would be likely to get some auscultatory finding at the left sternal border. Now, admittedly, in an interauricular septal defect sometimes one has a paucity of findings, but with a ventricular septal defect we ought to get a loud systolic murmur of some sort at the left sternal border. This patient did not have such a murmur, so it is unlikely that an interventricular defect was present. I also feel that mitral stenosis would have been the most likely diagnosis clinically prior to the findings obtained at catheterization.

DR. ALEXANDER: Dr. Bercu, I think my remarks will not be nearly as valuable as yours in the discussion of this situation. You wrote a note on the chart which stated that this was probably not mitral stenosis because the pulmonary capillary pressure was normal. Dr. Massie felt that in the absence of a louder murmur than was heard at the left sternal border an interventricular defect was unlikely. Did you feel that you had ruled this out by catheterization?

DR. B. BERCU: It is impossible to rule out septal defect with certainty when the pressure on the right side of the heart rises abnormally. Depending upon the pressure differential between the two ventricles and the size of the defect, one can miss a small defect with a left to right shunt, or a larger defect as the pressure on the right side rises and the shunt reverses, so that probably many cases in the literature cited as primary pulmonary hypertension really are septal defects without enough shunt to be obvious on catheterization. There is one other technic in using the catheter that might be worth mentioning. Unfortunately, we were not employing it at that time. It consists of injecting dye into the area of the shunt and measuring the time it takes for it to appear in a peripheral artery. Had we done that at the time we could have been more certain.

DR. ALEXANDER: You said that you did not think there was a left to right shunt. Why?

DR. BERCU: I did not think so because the oxygen content of the blood samples taken from the right side of the heart showed no significant rise as we went from the superior vena cava to the pulmonary artery. All the values were normal.

DR. ALEXANDER: If there were a left to right shunt would there have been more oxygenation of the right ventricular blood?

DR. BERCU: Yes.

DR. ALEXANDER: Pulmonary stenosis was suggested as a possible diagnosis here. How may catheterization rule this out?

DR. BERCU: That is the easiest diagnosis to rule out by catheterization, because if the systolic pressure in the pulmonary artery is equal to that in the right ventricle there is no doubt that pulmonary stenosis is absent. In the presence of pulmonary stenosis, the pressure in the pulmonary artery is markedly diminished as compared to that of the right ventricle.

DR. MASSIE: Since what you said rules out pulmonary stenosis, what about pulmonary insufficiency on the basis of the catheterization data?

DR. BERCU: You're probably thinking of the wide pulse pressure, Dr. Massie, which suggests that diagnosis. It is impossible to be certain about the presence of pulmonary insufficiency. In the presence of this type of pressure recording and of a high-pitched diastolic murmur heard along the left sternal border, which this patient

did not have, I would be willing to make a diagnosis of pulmonary insufficiency.

DR. MASSIE: In other words, your readings would be compatible with pulmonary insufficiency.

DR. BERCU: Yes.

DR. ALEXANDER: Would you get a loud P_2 in pulmonary insufficiency?

DR. BERCU: In the absence of pulmonary stenosis, yes, because the pulmonary insufficiency must be secondary to pulmonary hypertension.

DR. ALEXANDER: What is the mechanism of the murmur?

DR. BERCU: The valves probably close with a loud bang, but there is just enough space between them for some blood to leak back.

DR. ALEXANDER: Any other question?

DR. JOHN R. SMITH: Is the diastolic pressure of 45 mm. Hg in the pulmonary artery compatible with a wide-open pulmonary insufficiency?

DR. BERCU: I was speaking of the usual pulmonary insufficiency, which is a functional lesion secondary to pulmonary hypertension and dynamically probably not too important. If you are speaking in terms of dynamics and the effect on the right ventricle, I would agree with you that a high diastolic pressure in the pulmonary artery would be unlikely in pulmonary insufficiency.

DR. JAMES W. OWEN: The important thing is that neither a septal lesion nor a pulmonary insufficiency can explain the primary pathology here, which was right-sided hypertension.

DR. BERCU: That is not entirely true. Although a septal defect *per se* does not cause right-sided hypertension, about 10 per cent of those patients with either an auricular or a ventricular septal defect ultimately develop severe pulmonary hypertension. We also know that in the Eisenmenger complex pulmonary hypertension is a part of the lesion, so that diagnosis should have been considered.

DR. ALEXANDER: The absence of any definite lesion of the heart, the valves or the septum that may have accounted for this picture led to the consideration that the resistance might be in the pulmonary artery, and so a suggestion was made that this might be primary or perhaps secondary pulmonary artery hypertension. I believe, Dr. Wood, you made that diagnosis before the catheterization studies were done. I wonder if you'd tell us your concept of primary pulmonary artery hypertension.

DR. W. BARRY WOOD, JR.: Dr. Alexander, I suggested that diagnosis because I couldn't see the left auricular enlargement in the x-ray films. In addition, the patient had had trouble at the time of her pregnancy, which suggested the possibility of thrombi in the pulmonary circuit. Since all our evidence pointed to right-sided heart strain and pulmonary hypertension, it seemed to me that she might be suffering from a block in the lesser circulation, that is, in the pulmonary vessels, which was throwing a strain on the right ventricle, causing the very loud second pulmonic sound, and leading to her symptoms.

DR. ALEXANDER: The block, then, would be proximal to the pulmonary capillaries?

DR. WOOD: Yes, and the fact that the pulmonary arteries at the hilar region were not enlarged, as Dr. Seaman pointed out, raises the question as to whether the thrombi might not have been fairly close to the pulmonary conus involving the large pulmonary arteries.

DR. ALEXANDER: I am not very clear as to whether you are now speaking of so-called primary pulmonary hypertension or whether you feel there will be thrombi or a lesion somewhere in the lungs.

DR. WOOD: I was thinking in terms of multiple pulmonary thrombi leading to pulmonary hypertension. In those cases that I know about, the pathologist can demonstrate changes in the pulmonary blood vessels, either marked sclerosis of the small pulmonary arteries or thrombi that have become recanalized. There must be something producing a block, either in terms of sclerosis of the vessel walls or thrombotic changes inside the vessels. Those are the only changes with which I am familiar.

DR. ALEXANDER: This would be analagous, then, to systemic hypertension.

DR. WOOD: In a sense, yes.

DR. ALEXANDER: Dr. Owen, you're interested in this subject. Do you agree with this suggestion?

DR. JAMES W. OWEN: Yes, Dr. Alexander. As a matter of fact an increasing number of these cases is being discovered now that cardiac catheterization is being done with increased frequency, and both mechanisms Dr. Wood has described have been offered to explain various cases. It is interesting that studies of the pathology of the lung show that most of the changes have been limited to vessels having a diameter of 100 micra or greater. In the differential diagnosis it is important to distinguish this kind of cor

pulmonale from that secondary to chronic lung disease. Fortunately this woman is certainly one of a small number of women who have trouble during pregnancy and run a rapidly fatal course with progressive signs of cor pulmonale. The other interesting features that these patients almost uniformly demonstrate are the absence of orthopnea and the presence early in the course of their illness of normal oxygenation of arterial blood.

DR. ALEXANDER: In my earlier medical experience, this diagnosis meant that nothing was found at autopsy. Later on, more careful study of these cases did reveal minute healed thrombi or other larger thrombi. When we speak of primary pulmonary hypertension, we might be aware of the fact that this isn't necessarily primary. Dr. Bercu, how did you interpret her polycythemia?

DR. BERCU: I had great difficulty interpreting it, Dr. Alexander, for this reason: On two occasions we did determinations of her arterial oxygen content and saturation; in both of those instances we found that the arterial oxygen data were perfectly normal. Our usual concept of secondary polycythemia is that it occurs on the basis of anoxia or hypoxia, with a diminished arterial oxygen concentration and conceivably a diminished oxygen concentration in the bone marrow. If this is so, this patient did not have secondary polycythemia. On the other hand, the fact that pulmonary disease is often associated with pulmonary hypertension and with polycythemia would lead one to call this a secondary polycythemia. It is conceivable that she had primary polycythemia, but I would think this very unlikely.

DR. ALEXANDER: May we assume that the lesion is not in the pulmonary capillaries? If this were early Ayerza's disease, wouldn't the pulmonary capillary pressures be raised even in the earlier stages?

DR. BERCU: I am not sure of that. It is my impression that pulmonary capillary pressure would not be elevated unless there was disease of the left ventricle or the mitral valve.

DR. ALEXANDER: But if there were thrombi or fibrosis in the pulmonary capillaries, shouldn't one see eventually some evidence in the chest x-rays and shouldn't the capillary pressure be increased?

DR. BERCU: Changes could have taken place in the alveolar walls which would have nothing to do with the blood vessels, such as the interstitial change we see in an alveolar-capillary block which does not elevate capillary pressures. I'm not aware of any studies which show rises in the pulmonary capillary pressure from changes in the pulmonary capillaries themselves.

DR. ALEXANDER: Are there further questions about this case or shall we decide that, in all probability, this is a case of disease of the pulmonary artery itself? We are still a little uncertain about the polycythemia. Dr. Massie, do you agree with this?

DR. MASSIE: I am somewhat skeptical about the primary pulmonary vascular disease. I'm sure this patient didn't have mitral stenosis. Since we have ruled out that possibility, I wonder whether pulmonary insufficiency might have produced this type of polycythemia. I'm puzzled that we did not see more pulmonary vascular markings; this makes me hesitate to make a diagnosis of pulmonary insufficiency.

DR. ALEXANDER: You have made a very good point. Certainly in many of the cases that have been described, one of the features is a prominent pulmonary conus. Are there other questions or suggestions?

DR. MASSIE: I should say one thing about the electrocardiogram. There is no question that it showed marked right ventricular enlargement; we also made an electrocardiographic diagnosis of auricular enlargement, but it must be remembered that with pulmonary hypertension that kind of electrical pattern does appear, which does not necessarily require anatomic enlargement of the auricle. I agree that there was no left auricular enlargement on the x-ray films.

A PHYSICIAN: It is not safe to say there was no enlargement of the pulmonary conus. As Dr. Seaman pointed out, it is entirely probable that there may have been, especially on the right anterior oblique film. I would be inclined to say that the pathologist will tell us there is enlargement of the pulmonary conus.

DR. OWEN: Dr. Alexander, in regard to the polycythemia, I think it's worth mentioning that this is not a constant finding in this disease.

DR. ALEXANDER: We will turn this discussion over to the pathologists with a clinical diagnosis of primary pulmonary hypertension.

PATHOLOGIC DISCUSSION

DR. JOHN D. KEYE: The pleural cavities contained 250 ml. of a clear serous fluid in each hemithorax. The heart was markedly enlarged with dilatation of the right ventricle and atrium

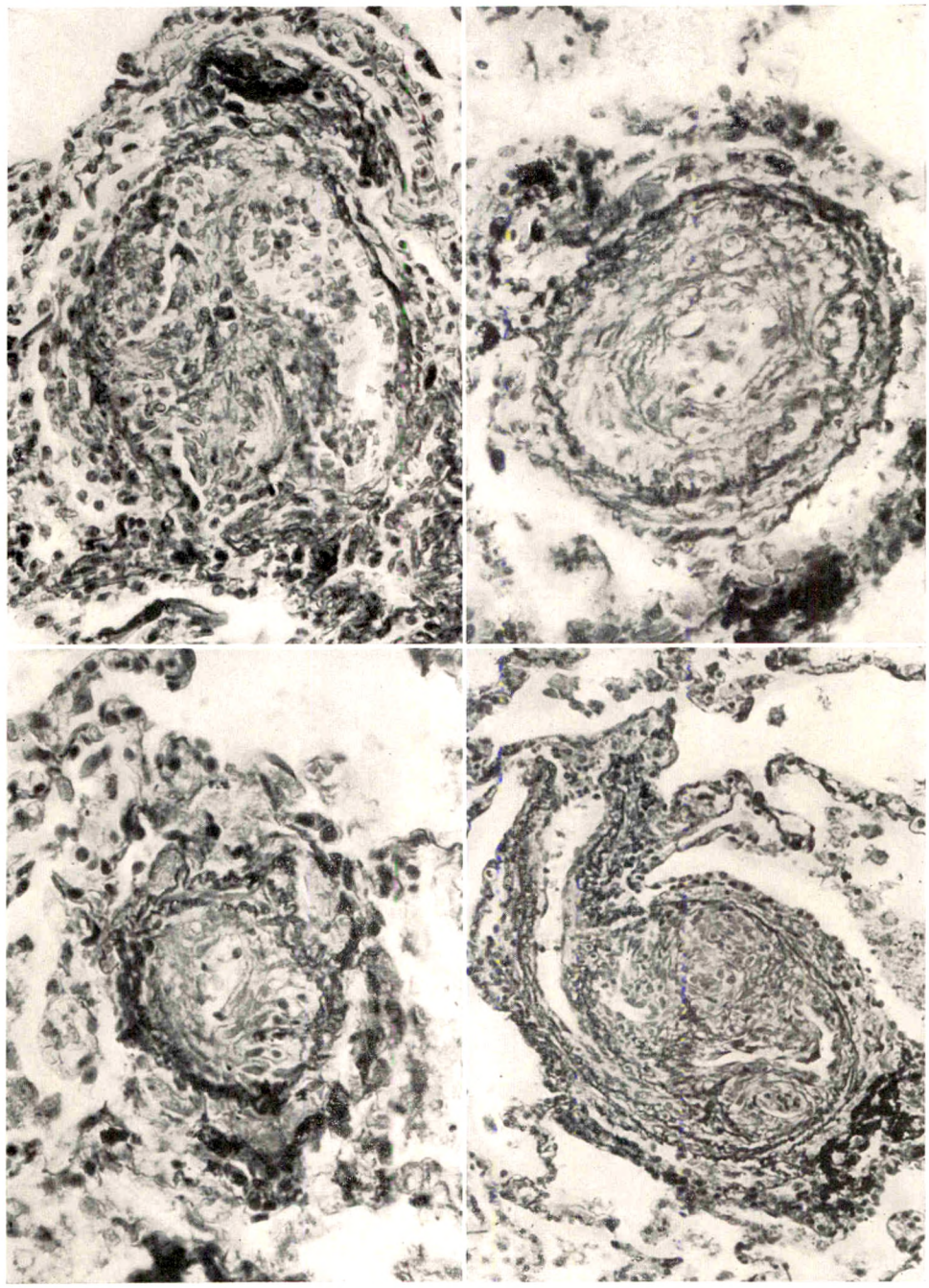


FIG. 1. Fibrous tissue and endothelial channels typical of an organized thrombus in a small pulmonary artery. This is one of two types of occlusive lesions that were present in tremendous numbers in the vessels of the lungs.

FIG. 2. Concentric fibrous intimal thickening in a small muscular artery (the second type of occlusive lesion of pulmonary arteries in this case).

FIG. 3. A lesion of the second type in an arteriole.

FIG. 4. Irregular intimal fibrous thickening in a terminal branch of an arteriole which suggests in its appearance a form intermediate between the obvious organized thrombi and the concentric lesions which are usually considered as arteriosclerotic.

and hypertrophy of their walls. The left ventricle and atrium were of more nearly normal configuration. The pulmonary valve, as well as all the other valves, was normal. The myocardium was not remarkable except for hypertrophy and the endocardium was thin and transparent. The thyroid was palpable but weighed only 24 gm.; upon section it did not appear unusual. The lungs were moderately edematous and congested. The large pulmonary arteries contained a few yellow plaques in the intima. The appearance of the liver was typical of advanced chronic passive congestion, and there was congestion of the spleen and of the kidneys. The veins of the legs, unfortunately, were not examined.

DR. WILBUR A. THOMAS: On microscopic examination all of the abdominal organs showed evidence of long standing congestive failure. Such changes were especially prominent in the liver, spleen and pancreas. The thyroid showed a few dilated acini but there was no evidence of increased activity. The lungs contained a few areas of a slight degree of congestion and edema, but there was no evidence of significant pulmonary fibrosis or emphysema. The significant pathologic changes in the lungs were limited to the small arteries and arterioles. Examination of these small vessels makes it apparent that the cause of the pulmonary hypertension in this patient was multiple obstructive lesions at this level.

These obstructive lesions were of two types. In the small pulmonary artery illustrated in Figure 1, it consists of a meshwork of loose connective tissue through which are scattered endothelial-line vascular channels. This is the usual and easily recognizable appearance of an organized thrombus, and on the left side of the figure it can be seen to bulge into a branch of the vessel. Figure 2 is an example of the second type of lesion, which consists of fibrous intimal thickening. This is a small muscular artery with a clearly defined muscular wall and internal elastica. The intima within the elastica is thickened by loose fibrous tissue that encroaches upon the lumen in a rather uniform fashion to reduce its size greatly. Figure 3 shows a similar lesion, this time in an arteriole. There are marked fibrous intimal thickening and a very small lumen. The lesion in Figure 4 is somewhat less advanced and considerably more irregular. It is in a terminal branch of a small muscular artery at the origin of an arteriole and its appearance suggests an intermediate stage between the first and second types of lesions. It is impossible to estimate the extent of these lesions. It is quite probable that there were literally millions of them. It can be readily appreciated that the degree of obstruction produced by large numbers of these lesions would be sufficient to result in pulmonary hypertension and eventually in right heart failure.

Our problem is to determine the basic nature of these lesions and, if possible, their etiology. Until very recent years, as Dr. Alexander pointed out, cases such as this probably would have been classified in most clinics and laboratories as primary pulmonary arteriosclerosis or Ayerza's syndrome. Ayerza's syndrome is a rather loosely defined term usually indicating any type of pulmonary arterial disease the cause of which is not immediately apparent. Considerable evidence has been acquired in recent years indicating that many of the cases previously called by this name are actually cases of latent pulmonary embolization with the lesions in pulmonary vessels resulting from repeated showers of small emboli. The evidence for this viewpoint comes from three sources, namely, animal experiments, autopsy observations and clinical observations. This is a very good example of the results that can be achieved with this combined approach.

Seven years ago a British pathologist, C. V. Harrison,[1] had a case similar to the present one. It occurred to him that these lesions might be due to emboli. In order to test this idea, he embolized a group of young, healthy rabbits by repeatedly injecting a saline suspension of blood clots into a peripheral vein and allowing them to be carried by the blood stream to the lungs. By means of this simple method, he was able to produce lesions like those seen in his human case and in the case being discussed here. His results have been confirmed by investigators in other laboratories throughout the world and we have duplicated them in some recent experiments of our own. Reports of animal experiments such as those by Wartman and others,[2] and Barnard,[3] as well as that of Harrison

[1] HARRISON, C. V. Experimental pulmonary arteriosclerosis. *J. Path. & Bact.*, 60: 289, 1948.

[2] WARTMAN, W. B., JENNINGS, R. B. and HUDSON, B. Experimental arterial disease: the reaction of the pulmonary artery to minute emboli of blood clot. *Circulation*, 4: 747, 1951.

[3] BARNARD, P. J. Pulmonary arteriosclerosis and cor pulmonale due to recurrent thromboembolism. *Circulation*, 10: 343, 1954.

include photomicrographs of lesions that are indistinguishable from the two types shown in the present case. The second type of lesion, the fibrous intimal thickening, may or may not necessarily be another form of organization of thrombi. It may be due to the hypertension that has been produced by the obstruction from lesions of the first type, which are easily recognized as organized thrombi. In any event, both types of lesions are readily produced by injecting emboli of blood clots into experimental animals.

In recent years, increasing numbers of human cases have been reported with pulmonary vascular lesions similar to those in the present case and associated with clearly demonstrable sources of emboli in the veins of the legs or the right portion of the heart. Several years ago we studied the autopsies of twelve such cases at the Massachusetts General Hospital.[4] Lesions of both types, recanalized thrombi and typical arteriosclerosis, were constantly present in the arteries of the lungs. In nine of these twelve cases there were clearly demonstrable sources in the leg veins or heart, usually in the leg veins, from which emboli could have arisen. In two cases the leg veins had not been examined because of failure to recognize their importance at the time of autopsy. In only one case no source of emboli was found despite a complete examination.

Supporting evidence for this concept of the etiologic importance of pulmonary emboli in these cases comes from clinical observations. Careful histories have revealed in some cases, although by no means in all, episodes which were suggestive of embolization. A number of cases have also been reported with onset dating from pregnancy, a condition of which thrombotic and embolic episodes have long been recognized as complications.

In summary, this is a case of chronic cor pulmonale resulting from multiple obstructive lesions in the small pulmonary arteries and arterioles. Evidence has been derived from experimental animals, autopsies and clinical observations indicating that these lesions may have resulted from multiple emboli. The source of emboli in this case was not demonstrated, but the veins of the legs, which are the most common source, were not examined because of failure to recognize the possibility at the time of the autopsy that they might have been important in clarifying the pulmonary condition. The immediate cause of death was not apparent, but in a number of the previously studied cases the terminal episode has also been rather sudden.

Final Anatomic Diagnoses: Multiple organized thrombi in the small pulmonary arteries and arterioles; slight arteriosclerosis of large pulmonary arteries and advanced arteriosclerosis of small pulmonary arteries and arterioles; myocardial hypertrophy and dilatation of the right ventricle and atrium.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

[4] OWEN, W. R., THOMAS, W. A., CASTLEMAN, B. and BLAND, E. F. Unrecognized emboli to the lungs with subsequent cor pulmonale. *New England J. Med.*, 249: 919, 1953.

Research Society Abstracts

Southern Society for Clinical Research

ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING, NEW ORLEANS, LOUISIANA, JANUARY 29, 1955

PHYSIOLOGIC VARIATIONS DURING INDUCED HYPOTHERMIA IN THE DOG. *H. M. Ausherman, W. K. Nowill, S. Boyarsky, H. E. Hall and W. W. Shingleton (introduced by J. V. Warren*)*. Dept. of Anesthesia, V. A. Hospital, Div. of Anesthesia and Urology and the Dept. of Surgery, Duke University Hospital and School of Medicine, Durham, N. C.

Recent enthusiasm for the use of induced hypothermia for cardiac surgery and the hibernation technic for severe infection and other shock-like states prompted the authors to familiarize themselves with the technics and physiologic variations of induced hypothermia in the dog. Hypothermia was produced most rapidly and efficiently by emersion in ice water under pentothal® or nembutal® sodium anesthesia. Biologic functions tested were as follows: blood pressure, pulse rate and electrocardiographic changes, respiratory rate, tidal and minute volume, oxygen consumption, electroencephalographic patterns, serum potassium and sodium, arterial pH, carbon dioxide tension, per cent oxygen saturation, hematocrit, renal and hepatic functions.

Respiratory rate, minute volume and oxygen consumption are markedly depressed. Arterial carbon dioxide pressure and pH vary toward respiratory alkalosis. Arterial blood remains well oxygenated until respiration is nearly absent. Serum sodium was essentially unchanged but serum potassium was decreased. Blood pressure and pulse rate declined progressively with the decrease in rectal temperature. Electrocardiographic changes were those associated with a sinus bradycardia and alkalosis.

Hypothermia in the dog appears to be a relatively safe procedure at body temperatures at or above 25°C. and for less than twelve hours. Temperatures below 25°C. and for more than twelve hours have a progressively higher mortality.

* Asterisks indicate members.

DACTIL (N-ETHYL-3 PIPERIDYL-DIPHENYL-ACETATE HYDROCHLORIDE) IN TREATMENT OF VENTRICULAR ARRHYTHMIA INDUCED BY OUABAIN. *D. P. Baumann and L. D. Seager (introduced by P. L. Day*)*. Univ. of Arkansas School of Medicine, Little Rock, Ark.

Recent attempts to find an effective antifibrillary agent for use in the arrhythmia of hypothermia has led to the trial of many drugs with indifferent results. Studies with dactil® (Lakeside Laboratories, Inc.) have suggested its applicability in this regard. Its effectiveness in the prevention of ventricular fibrillation due to benzol and epinephrine in hypothermic and normothermic dogs has been presented in a preliminary report elsewhere. As another phase of this study ventricular arrhythmia was induced by intravenous ouabain in pentobarbital anesthetized dogs. Dactil was administered in a slow, continuous intravenous drip until definite results were determined. Intermittent electrocardiographic tracings were obtained as necessary as a criterion of the status of the rhythm. Amounts of ouabain required to produce a ventricular rhythm varied considerably (0.07 to 0.395 mg./kg.). Dactil was given as a 0.5 to 1.0 per cent saline solution 10 to 50 mg. per minute. Reversion to a supraventricular or normal sinus rhythm occurred in fifteen of twenty experimental procedures completed to date. Present evidence suggests this drug may act in part by depressing ventricular irritability.

SERUM LIPID FRACTIONS IN INDUCED AND SPONTANEOUS HYPOTHYROIDISM; MODIFICATION BY BETA-SITOSTEROL. *M. M. Best,* C. H. Duncan, J. D. Wathen and H. D. Kerman.** Inst. of Medical Research, Univ. of Louisville School of Medicine, Louisville, Ky.

Serum lipid fractions were followed in a group of patients with congestive failure rendered hypothyroid by I-131. Four patients with rheumatic mitral stenosis had initially

low serum total cholesterol values (mean 146, range 109–180). Following I-131 administration they exhibited a transient peak in serum cholesterol during the second month (mean 196, range 181–215). In three of these patients the serum cholesterol followed a similar course, returning four months after treatment to the initial range, while the I-131 uptake remained in the hypothyroid range. For these subjects the mean values were: pre-treatment, 135, second month peak, 189, and return to 147 while the subject was still hypothyroid. In the fourth patient persistent hypothyroidism was associated with an eventual sustained increase in serum cholesterol, from pre-treatment level of 180 to 259 six months later.

Administration of the plant sterol, beta-sitosterol, to this patient and to patients with spontaneous myxedema resulted in a mean reduction of serum cholesterol of 25.9 per cent, comparable to that we have observed from sitosterol administration to euthyroid subjects.

The changes in cholesterol were paralleled by those in serum total lipid and, less consistently, by those in lipid phosphorus and calculated neutral fat.

CLINICAL AND PHYSIOLOGIC EVALUATION OF SEVERAL ORALLY EFFECTIVE BRONCHODILATORS. *H. G. Boren, D. J. George, I. C. Chafnas and C. A. Handley (introduced by C. L. Spurr*)*. V. A. Hospital and Dept. of Medicine of Baylor Univ. College of Medicine, Houston, Tex.

Three orally administered N-substituted arterenol derivatives have been evaluated. Twenty-four male patients, averaging fifty-eight years in age, with continuing difficulty from pulmonary emphysema and chronic bronchitis despite the usual regimen, were studied with special emphasis on pulmonary function. Toxic effects were limited to occasional slight decrease of blood pressure and tachycardia accompanied at times by palpitation or dizziness. No laboratory evidence of hematologic, hepatic, electrocardiographic or renal damage was found, even when drugs were administered over two months. Moderate to marked increase of vital capacity was frequently found. Timed vital capacity, nitrogen washout and flowmeter tracings added no further information.

For study of mechanics, pressure was measured by an intra-esophageal catheter, flow by Lilly flowmeter, volume by electrically recording spirometer. Changes were recorded on multi-channel electrocardiograph and oscilloscope. Mechanics studies on eight patients showed increase frequency of breathing only once, decrease of extremes of pressure fluctuation in seven of eight cases, volume and flow increase in three of four cases, and significant decrease of resistance in three of four patients. Compliance studies in five patients showed an increase twice and decrease once. Of the three drugs studied, N-(2-(3,4-methylenedioxyphenylisopropyl)) norepinephrine hydrochloride (JB251, Lakeside) is most effective. Only mechanical studies allow complete evaluation.

CORRELATION OF PHYSIOLOGIC EFFECTS OF RADIOACTIVE ENDOTOXIN WITH ITS DISTRIBUTION IN VIVO. *A. I. Braude, F. J. Carey, D. W. Sutherland and M. Zalesky (introduced by E. Strauss*)*. Dept. of Internal Medicine, Southwestern Medical School of the Univ. of Texas, Dallas, Tex.

As a means of investigating the pathogenesis of gram-negative bacterial infections, the fate of radioactive endotoxin, labelled with Cr^{51}, was correlated with its physiologic effects after intravenous injection. Endotoxin was prepared by culturing Escherichia coli in the presence only of inorganic constituents to avoid organic impurities which might bind chromium. Firm labelling was achieved by adding $Na_2Cr^{51}O_4$ or $Cr^{51}Cl_3$ directly to endotoxin or living E. coli and radioactivity was directly proportional to toxicity.

Labelled endotoxin, after intravenous injection of lethal doses in rabbits, first appeared in the plasma (but not in the erythrocytes) and then rapidly passed to buffy coat and liver. Tremendous concentrations accumulated in the buffy coat while leukopenia was developing. Rapid removal from plasma was accompanied by fever, leukopenia and sometimes hypotension and diarrhea. Changes in blood volume, determined with Cr^{51} labelled erythrocytes, were variable but not marked.

These results indicate that changes in leukocytes (neutropenia, toxic granulations, reduced mobility) after injection of endotoxin result from localization in them of endotoxin; that localization represents an active removal of endotoxin from the circulation; and that protection against endotoxin, as protection against bacterial infection, depends in part on the ability of leukocytes to ingest endotoxin.

NUCLEOTIDE LEVELS IN HUMAN CARDIAC MUSCLE. *W. J. Burdette.** Chester Beatty Research Inst. of the Royal Cancer Hospital and the Brompton Hospital, London, England.

Fresh, auricular muscle was obtained from eight patients at the time of cardiotomy for mitral stenosis and was placed on ice immediately. After weighing, the myocardium was homogenized and an acid extract prepared. Dowex-1 (200 to 400 mesh) was then used as a formate column to separate the nucleotides. The fractions were collected in volumes of 5 ml., the optical densities of which were read at 260 and 275 mμ in a Beckman model DU spectrophotometer. The results were rather uniform for the various specimens. The average amount (μM/gm.) of AMP was 0.721, of ADP was 0.292, and of ATP was 0.138. Complete separation of other nucleotides was not accomplished but their presence suggests that the adenosine system may not solely be involved in energy exchanges during muscular contraction of the human heart.

CASE OF COLLAGEN DISEASE (SCLERODERMA) IN WHICH PLEUROPNEUMONIA-LIKE ORGANISMS WERE ISOLATED FROM AN OVARIAN CYST. *S. W. Bush, L. B. Robinson, W. E. C. Wacker and T. M. Brown.** Dept. of Medicine, George Washington Univ. School of Medicine, and the Arthritis Research Unit, Medical Service, V. A. Hospital, Washington, D. C.

The case is that of a twenty-nine year old woman with repeated genitourinary infections for five years treated with penicillin. The present illness began five months before hospitalization. Clinical and laboratory studies were compatible with disseminated lupus erythematosus but no L.E. cells were found. Recurrent Escherichia coli urinary infections were treated with antibiotics other than penicillin. Cortisone was employed intermittently for symptomatic control. Laparotomy disclosed a follicular ovarian cyst. The clear cystic fluid contained pleuropneumonia-like organisms in pure culture. Improvement after surgery was followed by a downhill course. Treatment consisted of cardiac management and cortisone therapy. Autopsy, after fifteen months of illness, revealed findings compatible with scleroderma in the skin, gastrointestinal tract, lungs and heart.

This is the first case in which pleuropneumonia-like organisms (L organisms) were obtained from an ovarian cyst. These findings are interesting in view of the long-standing speculation regarding the role of pleuropneumonia-like organisms in Reiter's disease, genitourinary infections, rheumatoid arthritis and rheumatic fever. Also, the findings may pertain to the known ability of penicillin to induce the formation of 'L' forms from common bacteria. No conclusions regarding the etiology of collagen diseases can be drawn from an isolated case but additional studies appear indicated.

THE LEUKOPENIA OF VIRUS INFECTION—RELATION TO THE CONCENTRATION OF VIRUS AND THE VIRAL LESION. *G. R. Cary, W. B. Sorrell and E. D. Kilbourne.** Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La.

The well known leukopenia of virus infections is an enigmatic phenomenon which has received little study. With the belief that investigations on the genesis of this leukopenia might aid in elucidation of the pathogenesis of viral disease, a study was undertaken of experimental influenza virus infection. Adult male CFW mice weighing 20–22 gm. were inoculated intranasally with either 10^6 EID_{50} of influenza A virus (PR8 strain) or a control injection of saline. Both inocula contained penicillin and streptomycin. At varying intervals following infection (twelve, twenty-four, forty-eight, seventy-two and ninety-six hours) mice (four/group) were bled from the tail, then killed for study of pulmonary lesions, pulmonary virus concentrations and bone marrow cytology.

A 22 per cent decrease in the total leukocyte counts of virus-infected mice was noted forty-seven to ninety-six hours after inoculation. Differential counts revealed an absolute lymphopenia representing a 40–45 per cent reduction in lymphocytes and a concomitant, absolute neutrophilia. Leukopenia occurred twenty-four hours *subsequent* to the logarithmic phase of viral increase, *coincident* with the attainment of maximal viral concentration, and twenty-four hours *prior* to the appearance of pneumonia. Thus infection of a murine host with influenza virus results in an absolute lymphopenia and an absolute neutrophilia temporally related to the peak of virus increase but prior to the appearance of the gross viral lesion.

VASOACTIVITY OF AZIDES IN HYPERTENSION. *P. Comens (introduced by H. A. Schroeder**).* Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.

Utilizing photoelectric plethysmographs, photographic recordings were made in dogs after

intraarterial injections of choline azide (0.4 mg./kg.) and sodium azide (0.1 mg./kg.). Both produced unilateral local vasodilatation in the extremities of ten normotensive dogs, more marked with choline azide. A mean drop in diastolic pressure of 33 mm. Hg at fifteen minutes for both was more prolonged with the choline salt. Toxic doses of the latter (4.0 mg./kg.) caused marked electrocardiographic abnormalities. Two unanesthetized renal hypertensive dogs with blood pressures of 250/150 and 300/160 mm. received ten daily oral doses of choline azide; mean diastolic pressures dropped 44 to 64 mm. Hg for as long as five hours with recovery in twenty-four hours. When given intravenously, both drugs caused negligible late depressor effects in anesthetized normotensive rats. However, some operated animals showed significant diastolic depression only with choline azide. This agent administered gastrically caused insignificant changes in normotensive but marked prolonged depression in hypertensive rats. Neither agent blocked the pressor action of nor-epinephrine *in vivo*, although by the rabbit aorta strip method of Furchgott 0.05 mg. choline azide blocked the action of 2.0 γ nor-epinephrine. Sodium azide (5.0 mg.) used for long periods in four hypertensive patients at four-hourly intervals caused little or no effect upon blood pressure. These results suggest that choline azide is a long acting vasodilator when given orally to rats and dogs.

EFFECT OF L-TRIIODOTHYRONINE ON PROTEIN SYNTHESIS RATE MEASURED WITH N^{15} GLYCINE IN PATIENTS WITH PRIMARY MYXEDEMA. *K. R. Crispell,* W. Parson* and G. F. Hollifield.* Dept. of Internal Medicine, Univ. of Virginia School of Medicine, Charlottesville, Va.

The amino acid pool and the protein synthesis rate has been determined in five patients with primary myxedema using isotopic nitrogen, N^{15}. The patients were studied under the usual metabolic regimen. An interesting but unexplained finding was a moderate degree of positive nitrogen balance as determined by classic nitrogen studies. However, the protein synthesis rate as determined by the isotopic technic was consistently decreased in patients with myxedema. The average in the five patients was 379 mg./kg. per twenty-four hours (range 180–490) as compared with an average of 756 mg./kg. per twenty-four hours in five healthy volunteers (range 610–970). The size of the amino acid pool was not consistently altered.

Studies will be presented which show that several days after first administering L-triiodothyronine there is no increase in the protein synthesis rate. However, after administering the L-triiodothyronine for six to nine months the protein synthesis rate was markedly increased. For example, in one patient there was an increase in protein synthesis rate from 350 mg./kg. per twenty-four hours to a high of 1,300 mg./kg. per twenty-four hours.

HYPERACTIVITY OF THE BLOOD COAGULABILITY CONTROL MECHANISM—A POSSIBLE CAUSE OF IRREVERSIBLE SHOCK. *J. W. Crowell and W. L. Read (introduced by A. C. Guyton*).* Dept. of Physiology and Biophysics, School of Medicine, Univ. of Mississippi, University, Miss.

If an animal is bled until his arterial pressure is very low and this pressure level is maintained for a long period of time, a slowly falling blood pressure develops which continues to fall although the withdrawn blood is reinjected. Transfusion of additional blood or injection of sympathomimetic drugs has only a temporary effect, for the animal eventually dies. This is a type of irreversible shock.

Experiments in our laboratory have shown that concurrent with the fall in arterial pressure there is also a fall in the coagulation time of the circulating blood and that this hypercoagulability predisposes to the formation of large numbers of very small blood clots. If hypotension continues long enough for a major portion of the lung capillaries to be blocked, then the pulmonary resistance becomes so great that adequate circulation cannot be maintained. By use of large doses of heparin given before the beginning of the period of hypotension, the mortality rate from the hemorrhagic shock experiment is greatly lessened. Only one of ten dogs given heparin developed irreversible shock as compared with nine of ten dogs given no heparin.

CLINICAL RESULTS WITH PENTOLINIUM IN TREATMENT OF HYPERTENSION; COMPARISON WITH HEXAMETHONIUM WHEN BOTH ARE USED IN COMBINATION WITH RAUWOLFIA. *E. Dennis, R. Ford, W. Hughes, R. Hershberger and J. H. Moyer.** Baylor Univ. College of Medicine, Houston, Tex.

Recent reports by Freis and his co-workers have indicated that pentolinium is superior to other anti-hypertensive agents. In order to

evaluate the relative effectiveness of this agent, two groups of hypertensive patients were studied. Following adequate control observations, fifteen patients in Group I were given a combination of Rauwolfia and pentolinium. Group II was composed of sixty patients who received a combination of Rauwolfia and hexamethonium as therapy for six months to a year; these patients were then given a combination of Rauwolfia and pentolinium and the response rate, side reactions and tolerability of the drugs were compared. The results indicate that the small group of fifteen patients started on pentolinium and Rauwolfia (Group I) showed a 100 per cent response; 75 per cent of the patients in Group II responded to Rauwolfia in combination with hexamethonium; 73 per cent (forty-four patients) responded to Rauwolfia plus pentolinium; 10 per cent responded to neither combination; 15 per cent responded to Rauwolfia and pentolinium who had not previously responded to Rauwolfia and hexamethonium. On the other hand, 17 per cent who were previously responsive to Rauwolfia and hexamethonium were not responsive to Rauwolfia plus pentolinium. It is concluded that hexamethonium and pentolinium have a comparable effect in reducing the blood pressure when used in combination with Rauwolfia. The incidence and severity of side effects appears to be qualitatively similar but possibly less prolonged with pentolinium.

CORRELATION OF VENTRICULAR ELECTROKYMOGRAMS AND KINETOCARDIOGRAPHIC TRACES; APPLICATION TO STUDY OF MOVEMENTS OF THE HUMAN HEART. *E. E. Eddleman, Jr. (introduced by T. R. Harrison*)*. Dept. of Medicine, Medical College of Alabama, and Medical Service, V. A. Hospital, Birmingham, Ala.

Precordial chest wall movements (kinetocardiograms) were studied in a number of normal subjects as well as electrokymographic traces taken from many points of the ventricular borders. In addition simultaneous kinetocardiograms and electrokymograms were obtained in a few subjects. The similarities between the electrokymographic traces and the kinetocardiograms suggest that the movements of the precordium are primarily related to the movements and shape changes of the heart. By correlating the precordial movements with the electrokymographic traces from many points over the ventricular borders, a hypothesis for the movements of the normal heart can be arrived at: (1) anterior swing of the heart .02 second after the onset of the QRS complex, probably as the result of right ventricular contraction, (2) shortening of the heart and right ventricular inflow contraction, (3) apex thrust, possibly as the result of a physiologic aneurysm, (4) sphincteric squeeze of the entire ventricular walls, (5) elongation of the heart and inflow tract relaxation, (6) right ventricular relaxation with leftward and posterior swing of the heart, (7) filling and anterior rebound of the heart as the left ventricle relaxes.

BLOOD VOLUME IN PATIENTS WITH LAENNEC'S CIRRHOSIS AS DETERMINED BY RADIOACTIVE CHROMIUM TAGGED RED CELLS. *S. Eisenberg (introduced by A. Goth*)*. Dept. of Internal Medicine, Southwestern Medical School of the Univ. of Texas, and the Medical Service and Radioisotopes Unit of the V. A. Hospital, Dallas, Tex.

Twenty subjects with Laennec's cirrhosis were studied by the radiochromium tagged red cell technic. Nineteen patients had ascites; none had bled for a four-month period prior to the study. Comparisons were made between pre- and post-treatment values and between these and the values in a group of twenty normal persons.

Results: (1) Plasma volume was increased 41 per cent and blood volume 29 per cent in patients with esophageal varices and/or cyanosis; in the absence of these features these measurements were normal. (2) Despite lowered hemoglobin values, the red cell mass was normal in the twenty subjects studied suggesting that the "anemia of liver disease" may frequently be due to hemodilution. (3) In four cyanotic subjects the red cell mass was 49 per cent greater than normal. (4) Inconsistent changes in plasma volume followed clinical improvement and recession of ascites.

These findings suggest in the absence of evidences of an expanded vascular bed or a stimulus to erythropoiesis, the blood volume in Laennec's cirrhosis is normal; and "anemia of liver disease" is often a result of hemodilution.

EFFECT OF CEREBRAL CORTICAL STIMULATION IN CATS UPON EPINEPHRINE ACTIVITY AS INDICATED BY BLOOD LACTIC ACID LEVELS. *R. W. Ferguson, M. G. Mitts, M. Bernhaut and E. C. Hoff (introduced by J. L. Patterson, Jr.*)*. Dept. of Medicine and Neurologic Science, Medical College of Virginia, Richmond, Va.

The present studies were undertaken to determine whether stimulation of cerebral cortical areas, known to mediate autonomic responses, would also evoke increased adrenal medullary activity. The blood lactic acid concentration was used as the indicator of increased epinephrine secretion, since DiSalvo and colleagues (1953) demonstrated its validity for this purpose, in the absence of muscular movement or hypoxia. Acute experiments were performed under ether anesthesia, using d-tubocurarine and intermittent positive pressure breathing. Inductorium or rectangular-wave stimuli lasting ten seconds were delivered at intervals to loci on left and right anterior gyri alternately over a period of one hour with resulting transient hypertension, pupillary dilatation and nictitating membrane retraction. Blood lactic acid determinations made on fifteen stimulated cats and eleven unstimulated control animals (subjected to identical surgical procedures) showed a mean rise of 27 mg./100 ml. in the stimulated and 12.5 mg./100 ml. in the controls, a difference statistically significant ($p < .01$). Cats prepared surgically the day prior to the experiment in order to diminish stress showed lower initial lactate concentrations and greater increases with stimulation.

It is concluded that electrical stimulation of anterior sigmoid loci of the cerebral cortex causes increased secretion of epinephrine from the adrenal medulla.

CEREBRAL BLOOD FLOW, CARDIAC OUTPUT, CARDIAC WORK, MEAN ARTERIAL PRESSURE AND RIGHT HEART PRESSURE DURING POSTURAL SYNCOPE. *F. A. Finnerty, Jr., E. A. Partenope, J. F. Fazekas,* J. C. Rose and L. S. Lilienfield.* Dept. of Medicine, Georgetown Univ. School of Medicine and the Georgetown Univ. Medical Division, District of Columbia General Hospital, Washington, D. C.

In an attempt to clarify the pathogenesis of postural syncope cerebral and cardiac hemodynamic studies were performed in the 40° head-up tilt. Cerebral hypoxia was induced by lowering the arterial pressure acutely with intravenous hexamethonium and/or by posture. In the heterogeneous group of forty-six subjects studied, although the level of arterial pressure at time of syncope varied between 100.7 and 40.9 mm. Hg, signs of cerebral hypoxia developed when the cerebral blood flow was reduced to 31.5 cc./min./100 gm. brain (standard error 0.98). With the onset of cerebral hypoxia, right ventricular pressures decreased sharply, intracardiac pressure waves broadened and flattened, and direct Fick cardiac outputs measured in twelve subjects showed an average reduction of 42.6 per cent with a range of 20 to 68 per cent. Cardiac work was reduced by more than 70 per cent in all patients, which together with the production of bradycardia explained the absence of angina and electrocardiographic changes.

These observations are consistent with the concept that the mechanism of cerebral hypoxia was due to loss of vasoconstrictor tone which in these tilted subjects prompted failure of venous return, decrease in right ventricular pressure, decrease in cardiac output and finally, a decrease in cerebral blood flow below the critical level.

METABOLIC EFFECT OF QUINIDINE; EVIDENCE SUGGESTING IMPAIRMENT OF GLUCOSE UTILIZATION. *R. H. Furman* and R. P. Howard.** Cardiovascular and Endocrinology and Metabolism Sections of the Oklahoma Medical Research Foundation, and the Dept. of Medicine, Univ. of Oklahoma School of Medicine, Oklahoma City, Okla.

Since our initial report of nitrogen retention ("protein anabolic effect") during the administration of quinidine sulfate to human subjects, seven additional subjects have been studied. Complete metabolic balance studies were carried out in five, and significant nitrogen retention without salt or water retention was noted in four subjects.

Studies of carbohydrate metabolism were carried out in three subjects: a forty-two year old man with remote myocardial infarction and two half-brothers, aged ten and six, with Von Gierke's disease (hepatic only). Glucose tolerance curves during quinidine administration revealed significantly impaired tolerance when compared with control curves. Because of the possibility that quinidine may alter cell permeability to insulin, further tolerance studies are underway in additional subjects utilizing both glucose and fructose. According to Uyeki, Geiling and Dubois quinidine sulfate inhibits the oxidation of glucose, pyruvate and certain other substrates by rat heart slices, possibly by inhibiting transphosphorylating enzymes.

EXPERIMENTAL BLOOD COOLING TO 1.5°C. WITH A PUMP-OXYGENATOR FOR OPEN CARDIAC SURGERY. *F. Gollan (introduced by G. R. Meneely*).*

Research Laboratory and Radioisotope Unit, Thayer V. A. Hospital, Nashville, Tenn.

Cardiac surgery during hypothermia alone is limited by the short period of inflow occlusion and ventricular fibrillation; extracorporeal circulation has its drawback by the complexity of apparatus, the large coronary flow and the danger of air embolism. By a combination of the two approaches these difficulties were overcome. The entire venous return to the right heart was shunted into a pump-oxygenator, refrigerated and returned under pressure into the aorta. When satisfactory artificial oxygenation was achieved the respirator was turned off. At 13°C. the heart stopped beating and the left atrium or ventricle was opened. The heart chambers were empty and, due to cardiac arrest, coronary air embolism did not occur. After about thirty minutes of cardiac arrest the heart was closed, the blood was rapidly rewarmed in the extracorporeal circulation and normal cardiac and respiratory functions returned. The lowest body temperature reached was 1.5°C. Thirteen dogs survived this procedure without evidence of cerebral or cardiac damage. Four animals died of postoperative complications. Nine dogs were observed for one month.

RELATIONSHIP OF RIGHT ATRIAL PRESSURE TO CARDIAC OUTPUT FOLLOWING MYOCARDIAL DAMAGE. *A. C. Guyton,* A. W. Lindsey and B. N. Kaufmann.* Dept. of Physiology and Biophysics, Univ. of Mississippi School of Medicine, University, Miss.

In quantitative experiments on dogs, the following dual effects of right atrial pressure on cardiac output have been studied: Increasing the right atrial pressure increases the filling pressure in the heart itself and therefore increases the cardiac output; this is the well known Starling's law. Increasing the right atrial pressure also causes increased back pressure in the veins, which decreases the return of blood from the peripheral vessels to the heart. When these two opposing effects are equated, right atrial pressure automatically assumes a level which allows the venous return to the heart to equal the output from the heart. The quantity of blood pumped by the heart is determined not only by the filling pressure in the right ventricle but also by the integrity of the cardiac muscle itself, for a progressively damaged myocardium pumps progressively less blood at any given right atrial pressure. Increasing the blood volume increases the right atrial pressure and partially overcomes the diminished cardiac output due to myocardial damage. Graphs have been constructed to depict how varying degrees of myocardial damage and varying fluid volumes determine right atrial pressure and cardiac output simultaneously.

MALIGNANT HYPERTENSION: INFLUENCE OF RETINOPATHY, UREMIA AND UNDERLYING RENAL DISEASE ON MORTALITY AND SURVIVAL TIME. *J. A. Hagans, A. A. Brust* and E. B. Ferris.** Dept. of Internal Medicine, Emory Univ. School of Medicine and Grady Memorial Hospital, Atlanta, Ga.

Sixty-four patients with untreated malignant hypertension have been studied over an average observation period of four years. Duration of study was sufficient to evaluate the natural course since 88 per cent of the patients were dead at the close of the study.

The presence or absence of uremia was the only important factor in mortality. Grade 3 fundi, grade 4 fundi, underlying chronic renal disease or "essential" hypertension did not influence mortality figures. Mortality for the period of study was strikingly less in those without uremia at initial contact (70 per cent); all patients with uremia were dead. Mortality was similar in patients with grade 3 and grade 4 fundi (87 per cent and 88 per cent, respectively) but the grade 3 patients survived much longer (18.8 months) than the grade 4 (3.3 months). This difference was in patients without uremia as there was no difference in survival time of patients with grade 3 and 4 fundi who had uremia initially (all this holds true in both chronic renal and essential hypertensive groups). Uremic patients with chronic renal disease lived longer (6.4 months) than uremic patients in the essential hypertensive group (1.3 months).

HEMATOLOGIC STUDIES IN HEAT STROKE: THE ANEMIA OF HEAT STROKE WITH EMPHASIS ON A HEMOLYTIC COMPONENT. *E. R. Halden, F. Jones, D. A. Sutherland and E. E. Muirhead.** Depts. of Medicine and Pathology, The Univ. of Texas Southwestern Medical School, Dallas, Tex.

Fifteen patients with heat stroke were studied. The rectal temperature varied between 106–112°F. (10 between 108–112°F.). There were two age groups: seven aged thirty-two to fifty-four years (average, forty-six), eight aged sixty-three to ninety-seven years (average, seventy-five). The initial peripheral hemoglobin of 11.6–16 gm./

100 cc. (average 13.6) decreased within about one week to 8.2–13.8 gm. (average, 10.7). There was no obvious blood loss. By autotransfusion the survival of the patient's RBC was studied by the radiochromium method in thirteen patients. An increased rate of RBC destruction was detected in ten patients. The mean life-span values were: thirty to forty-five days for six, fifty to eighty days for four. Four-day stool urobilinogen in six patients averaged 242 mg. per day, giving a hemolytic index of about two times the upper limits of normal. Additional findings included: normal or slightly elevated WBC; slight reticulocytosis in five patients; thrombocytopenia in all (platelets < 100,000 in eleven); elevated ESR; elevated initial eosinophil count (680–1,300 per cu. mm.); prolonged clotting time in five patients; evidence of cell injury in the peripheral blood and bone marrow.

The anemia of heat stroke is partly due to a hemolytic state and partly due to injury to the bone marrow. It is plausible to consider the hemolysis related to the capillary stasis of this condition.

EFFECT OF RESERPINE ON THE HYPOTHALMIC PRESSOR RESPONSE. *F. Harrison and A. Goth.** Dept. of Anatomy, Pathology and Pharmacology, The Univ. of Texas Southwestern Medical School, Dallas, Tex.

The postulated hypothalamic site of action of reserpine was tested by direct electrical stimulation using the stereotaxic technic. Under chloralose urethane anesthesia, blood pressure was recorded in twelve cats. The hypothalamus was stimulated repeatedly before and after the injection of reserpine (100–200 μg./kg.). In addition, epinephrine or L-norepinephrine was injected repeatedly in order to estimate vascular responsiveness to the sympathetic mediators. The following effects were observed following reserpine: progressive increase in responsiveness to injected epinephrine, gradual hypotension, bradycardia, moderate to marked decrease in the height and alteration of the shape of the pressor response to hypothalamic stimulation. The hypothalamic pressor response, following the initial depression, returned in some experiments to normal or even above. In these cases, however, there was such an exaggeration of the pressor effect of injected adrenergic drugs that this phenomenon may be due to increased vascular responsiveness rather than the return to normal sensitivity of neural structures. In acute spinal animals in which there is already hypotension and increased responsiveness to epinephrine, reserpine causes no further such effects. These results support the hypothesis that reserpine depresses the hypothalamus or its descending sympathetic pathways.

SYSTOLIC MOVEMENTS OF THE PRECORDIUM AND OF THE BODY IN PATIENTS WITH BUNDLE BRANCH BLOCK. *T. R. Harrison,** *E. E. Eddleman and T. J. Reeves.* Dept. of Medicine, Medical College of Alabama, and the Medical Service of the V. A. Hospital, Birmingham, Ala.

In normal subjects there is a sharp inward movement of the parasternal region about .03 second before the onset of the carotid upstroke. This is thought to represent right ventricular ejection. In patients with right bundle branch block the time from the beginning of the QRS to the onset of the carotid upstroke is slightly prolonged while the time from the onset of the QRS to the sharp parasternal inward movement is more markedly prolonged. In patients with left bundle branch block the time from the ECG onset to right ventricular ejection appears to be slightly prolonged while the time elapsing between the onset of the QRS and the beginning of the carotid upstroke is markedly prolonged. It would appear that the contraction of each ventricle contributes somewhat to expulsion by the other ventricle. The onset of the G-H upstroke and of the H-I downstroke of the ballistocardiogram is markedly delayed in right bundle branch block but there is minimal or no delay in patients with left bundle branch block. This suggests that structures activated by the right bundle play a predominant role in the genesis of these headward-footward movements of the body. The precordial movements likewise tend to show alterations in the two conditions.

RENAL HEMODYNAMIC RESPONSE TO PENTAPYRROLIDINIUM; COMPARISON WITH HEXAMETHONIUM. *R. L. Hershberger (introduced by J. H. Moyer**).* Baylor Univ. College of Medicine, Houston, Tex.

Pentapyrrolidinium is a ganglionic blocking agent of the quaternary ammonium group structurally related to hexamethonium but believed to have fewer side effects. Since this agent has been reported to be superior to hexamethonium in the treatment of hypertension, a series of patients was studied for the effects of pentapyrrolidinium on renal hemodynamics in hypertension. Nine patients re-

ceived continuous intravenous infusions and eight received single intravenous doses during renal clearance studies. These were compared with previous studies using hexamethonium. It was shown that: (1) Pentapyrrolidinium produced a more pronounced and prolonged hypotension than that produced by hexamethonium. (2) After initial reduction in mean blood pressure by both continuous infusion and single injection of pentapyrrolidinium, glomerular filtration rate and renal blood flow were significantly depressed. Three hours after a single injection these functions increased significantly and approached control levels despite maintained hypotension. With continuous infusions, however, both GFR and RBF remained depressed. (3) Sodium and water excretion were depressed initially and remained depressed. (4) Potassium excretion was not significantly altered. The over-all effect of pentapyrrolidinium on renal hemodynamics was essentially the same as that found with intravenous hexamethonium. Differences found were a reflection of the greater reduction of pressure produced by pentapyrrolidinium.

ACUTE ELEVATION OF HEPATIC VENOUS AND ARTERIAL SERUM POTASSIUM DURING HYPERVENTILATION. *J. B. Hickam,* W. P. Wilson and R. Frayser.* Depts. of Medicine and Psychiatry, Duke Univ. School of Medicine, Durham, N. C.

In the first one to two minutes of voluntary hyperventilation there appears a shift of the arterial CO_2 dissociation curve in a direction which considerably accentuates the alkalinity of the serum. This proves to result from an acute rise in serum potassium, averaging 1.3 mEq./L. after two minutes of hyperventilation (twelve subjects). In looking for the source of this potassium it was found that jugular venous and peripheral venous potassium concentrations are slightly below arterial, but that hepatic venous serum has a much higher potassium content during hyperventilation than blood from any other site. In four subjects the mean increase in hepatic venous potassium was 2.3 mEq./L. The mechanism of this outpouring of potassium from the splanchnic region during early hyperventilation is not yet established.

This phenomenon accounts for about 25 per cent of the pH rise during early hyperventilation. It may well play a significant part in certain of the phenomena associated with hyperventilation, such as tetany, electrocardiographic changes, and possibly some of the acute vasomotor responses. The mechanism of this potassium rise may be related to that reported to occur when severe respiratory acidosis is abruptly relieved by overventilation, as in anesthesia during thoracic surgery.

METABOLIC AND PSYCHOLOGIC STUDIES ASSOCIATED WITH COLLEGE ROWING. *S. Richardson Hill, Jr., H. M. Fox, B. Murawski, S. J. Gray, J. R. St. Marc and G. W. Thorn (introduced by T. R. Harrison*).* Dept. of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Mass.

This study was designed in an attempt to clarify further the role of the adrenal cortex and various psychologic factors in the human response to stress. The subjects were members of a 1954 college varsity crew studied for six days during the final two weeks of preparation for the four-mile Harvard-Yale race. Eosinophil counts were taken at 9 A.M., 5 P.M. and 9 P.M. Four-hour urine specimens were collected from 9 A.M. to 9 P.M. and one specimen from 9 P.M. to 9 A.M. for determination of the 17-hydroxycorticoid, 17-ketosteroid and uropepsin excretion. Psychologic measurements included two Rorschach tests, individual Thematic Apperception Tests and personal interviews.

The eosinophil response showed a mean 20 per cent increase on the control day, a 30 per cent decrease on the regular practice day, a 69 per cent decrease on the time trial day and a 70 per cent decrease on the race day, over the same 5 to 9 P.M. period. The 17-hydroxycorticoid and 17-ketosteroid data revealed highly significant ($P < .01$) differences between individuals and between the race day as compared to the control and practice days, with no significant difference between values on the control day as compared with the practice day. On race day there was an obliteration ($P < .01$) of the daily diurnal decrease in 17-hydroxycorticoids. Certain correlations have been made between metabolic response and personality structure.

STUDY OF THE QUANTITATIVE RELATIONSHIP BETWEEN ANTIDIURETIC HORMONE AND RENAL TUBULAR REABSORPTION OF WATER. *W. Hollander, Jr., T. F. Williams, C. C. Fordham, III, and L. G. Welt.** Dept. of Medicine, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.

The rate of excretion of urine and the clearance of free water during physiologic diabetes

insipidus have been compared with those values observed during various constant rates of infusion of pitressin,® in healthy young adult males. A positive balance of water of approximately 1 L. was maintained throughout each study.

At relatively constant rates of solute excretion, the changes in the rates of excretion of urine (ΔV) and clearance of free water (ΔC_{H_2O}) are approximately equal and represent the increased rate of renal reabsorption of water resulting from the administered pitressin. At rates of infusion of pitressin between one and twenty milli-units per hour, the increase in rate of renal tubular reabsorption of water appears to vary as an exponential function of the rate of administration of pitressin according to the equation: $\Delta C_{H_2O} = 2.7$ (pitressin rate)$^{0.52}$. Although not yet established unequivocally, the generality of this or a similar relationship is suggested by the high degree of statistical significance with which the data fit this equation.

It is inferred that, in the range studied, the increase in renal tubular reabsorption of water promoted by pitressin is approximately a function of the square root of the rate of administration of pitressin.

ABSORPTION, TRANSPORT AND EXCRETION OF Cu^{64} IN THE NORMAL HUMAN AND IN PATIENTS WITH WILSON'S DISEASE. *W. N. Jensen and H. Kamin (introduced by T. B. Schwartz.*)* Depts. of Medicine and Biochemistry, Duke Univ. School of Medicine, and V. A. Hospital, Durham, N. C.

Non-isotopic copper studies have shown that there is a constant increase in brain and liver copper content, hypocupremia and hypercupuria in Wilson's disease.

Cu^{64} was administered orally to four normal subjects and three patients with Wilson's disease. Radiocopper content of blood, plasma, urine and feces was measured over a sixty-hour period. All normals had an initial peak of plasma non-globulin-bound radiocopper, a decline during the next six to eight hours; followed by a second, progressive increase present during the remainder of the study. The secondary rise consists of globulin-bound radiocopper. In Wilson's disease there was no secondary rise in total plasma or globulin-bound radiocopper. Urinary excretion correlated with the increased plasma non-globulin radiocopper. Fecal excretion of radiocopper was decreased.

It is suggested that copper is transported from the intestine to the liver; incorporated into a copper-globulin complex and released to the plasma. The initial peak of non-globulin-radiocopper represents leakage around the copper-globulin binding mechanisms when these are presented with increased quantities of copper. One defect in Wilson's disease consists of a metabolic block at the copper globulin synthesis and/or release stage. Other abnormalities in copper metabolism may be secondary to this defect.

ANALYSIS OF THE CHEMICAL EVENTS AND THEIR INTERRELATIONSHIPS WITH ALTERATIONS IN ECG DURING RESPIRATORY ACIDOSIS AND ALKALOSIS. *S. B. Joyner, D. A. Davis, D. T. Young, E. Craige and L. G. Welt.** Univ. of North Carolina School of Medicine, Chapel Hill, N. C.

The chemical alterations induced by respiring high concentrations of CO_2, and hyperventilation with room air, have been studied in three groups of dogs as follows: (1) simultaneous arterial and central venous blood, (2) simultaneous arterial, peripheral and hepatic venous blood, and (3) arterial blood samples in experiments conducted forty-eight hours following nephrectomy and injection with inulin. The effects of hypercapnia are: decrease in pH, increase in pCO_2, increase in inulin space, and an accession of sodium, potassium, glucose and phosphorus to the inulin space. The increment of sodium and potassium appears to be derived from the muscles, glucose from the liver (splanchnic area), and phosphorus from both. Hyperventilation promotes: an increase in pH, and a decrease in pCO_2, an increase in the inulin space is again observed, and larger increments of sodium and potassium, with smaller accessions of glucose, and inconstant increments of phosphorus to the inulin space. Sodium appears to leave muscle and liver, potassium is lost from liver but gained by muscle. The liver releases glucose, and both liver and muscle release phosphorus.

The alterations in the ECG are not consistently predictable in terms of pH, pCO_2 or the concentrations of potassium in serum.

RENAL SODIUM REGULATION: A COMPARISON OF THE EFFECT OF RECUMBENCY AND NECK COMPRESSION. *G. A. Kelser, Jr., H. L. Izlar, E. H. Estes, Jr. and J. V. Warren.** Dept. of Medicine, Duke Univ. School of Medicine, and V. A. Hospital, Durham, N. C.

Sodium excretion has been reported to increase upon changing from the sitting to the recumbent position and upon compressing the neck of normal sitting subjects. Observations have been made on eight healthy individuals to estimate the magnitude of the effect of these maneuvers. Hourly urinary sodium values were obtained on a control day with the subjects in a sitting position drinking 200 ml. of a 0.14 per cent sodium chloride loading solution every thirty minutes. The fifth through the eighth hours were designated test periods. In the test period on subsequent days either the horizontal position was assumed or a cuff was inflated around the neck to a pressure of 20 mm. Hg in the sitting position.

Recumbency produced a significant increase in sodium excretion in the seventh, eighth and ninth hours when compared with the control day ($p < 0.01$) and with the control period on the test day ($p < 0.01$). However, the values on the neck compression day were not significantly different from the control day ($p > 0.5$) or the control period on the test day ($p > 0.5$). It is concluded that this experimental design affords a valid method of investigation not only of the presence of sodium regulatory mechanisms but of their comparative importance.

EFFECTS OF ACTH AND ROTHANE ON THE RENAL CLEARANCE OF THIOSULFATE. *W. M. Kelsey, E. H. Yount, and J. M. Little.** Depts. of Pediatrics, Medicine and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

During the course of a study of the effect of ACTH and rothane on renal function in the dog 137 simultaneous measurements of C thiosulfate and C inulin were made under four circumstances: (1) control, (2) ACTH, (3) rothane and (4) rothane plus ACTH. ACTH increased the C thiosulfate and statistically altered the C thiosulfate C inulin ratio when compared to control values. Rothane lowered the C thiosulfate and also resulted in an altered C thiosulfate/C inulin. Rothane plus ACTH raised the C thiosulfate above control values but not to the level when ACTH was given alone. Pregnant dogs responded differently than nonpregnant animals when ACTH was given.

It is suggestive that in the dog the renal clearance of thiosulfate approximates the GFR but does not actually measure it since it is significantly less than the C inulin. During ACTH administration the C thiosulfate equals the C inulin. This suggests that ACTH decreases the renal tubular absorption of thiosulfate. Increase in the excretion of thiosulfate was not accompanied by detectable changes in electrolyte or water re-absorption.

EFFECTS OF ANGIOTONIN ON PULMONARY ARTERY PRESSURES OF ACUTE GOLDBLATT DOGS. *W. L. Kenoyer, L. G. May, A. Bennett, S. Y. Tsai and R. Gregory.** John Sealy Memorial Laboratory and Dept. of Internal Medicine, Univ. of Texas Medical Branch, Galveston, Tex.

Pulmonary artery pressures are normal in hypertensive patients. We have observed that this is true in dogs made chronically hypertensive by the Goldblatt method. It was further noted that angiotonin was capable of elevating pulmonary artery pressures as well as systemic pressures in normotensive and hypertensive patients and dogs.

Since long-standing renal hypertensive dogs may have normal pulmonary artery pressures due to some adaptive mechanism, it was thought necessary to make similar studies in dogs made acutely hypertensive. Seven dogs were made hypertensive by the Goldblatt technic. Pulmonary artery and systemic pressures were measured in these animals before and at intervals following the development of systemic hypertension. In no case was the pulmonary arterial pressure elevated. In six dogs angiotonin was given during the acute phase of systemic hypertension. Each dog responded to angiotonin with an elevation of pulmonary and a further elevation of systemic pressures.

These data do not support the concept that acute renal hypertension in dogs is due to angiotonin.

CONCENTRATION OF TETRACYCLINE IN THE SERUM FOLLOWING PARENTERAL ADMINISTRATION. *B. Kent, E. Shoemaker, E. Townsend, B. Bennett and E. M. Yow.** Baylor Univ. College of Medicine, and the Jefferson Davis and V. A. Hospitals, Houston, Tex.

This study was designed to evaluate the level of tetracycline attained in the serum, the time of peak levels and the duration of significant levels following parenteral administration of the antibiotic. Fifty patients were given either single intramuscular injections, single intravenous injections or intramuscular injections at eight-hour intervals over a five-day period. The doses

ranged between 50 mg. and 500 mg. of the antibiotic. An additional group of patients with either hepatic or renal impairment were given the antibiotic and were compared with the normal patients. Serum was collected at intervals over a twenty-four-hour period following single injections and daily in these patients receiving multiple injections. The concentration of the drug in the serum was determined by the twofold serial dilution method.

Following single intramuscular injections peak levels were reached in four hours, significant levels being sustained for eight hours following 100 mg. injections and twelve hours following larger doses. These levels were approximately twice as high as levels previously reported for equivalent oral doses. A tendency toward accumulation was noted only within 250 mg. multiple doses. High levels were attained by intravenous administration of the drug, however, these were of shorter duration than those following intramuscular injection.

RENAL RESPONSE TO AMMONIUM CHLORIDE ACIDOSIS IN GLYCOSURIC OSTEOMALACIA. *L. H. Kyle,* J. J. Canary and R. J. Meyer.* Dept. Medicine, Georgetown Univ. School of Medicine, Washington, D. C.

Renal tubular disorders associated with osteomalacia comprise two major groups. One is characterized by hyperchloremic acidosis, and the skeletal demineralization is attributed to cation loss secondary to impaired urinary acidification. The other group, known as the Fanconi syndrome, manifests glycosuria and other reabsorptive defects. Although acidosis may occur in this type of tubular insufficiency, its mechanism has received inadequate study.

Blood and urinary changes were followed during induction of ammonium chloride acidosis in three patients with glycosuric osteomalacia. The first patient, who had the infantile form of the disorder with cystinosis, showed a normal serum chloride, low bicarbonate and loss of bicarbonate in the urine. Acid loading caused no increase in ammonia production. The second patient demonstrated numerous reabsorptive defects and the chemical pattern of hyperchloremic acidosis. Minimal augmentation of urinary ammonia and titratable acidity resulted from administration of chloride. The third patient had glycosuria and hyperphosphaturia but the serum chloride and bicarbonate concentrations were normal. In this case, renal base-sparing mechanisms responded normally to administration of ammonium chloride.

These studies indicate that the two renal syndromes are not clearly separable and suggest the presence of factors other than cation loss in causation of osteomalacia.

EFFECT OF BLOOD TRANSFUSIONS IN PATIENTS WITH PERNICIOUS ANEMIA. *B. S. Leavell* and J. D. Mason, Jr.* Dept. of Internal Medicine, School of Medicine, Univ. of Virginia, Charlottesville, Va.

In 1946 Davidson et al. reported that in pernicious anemia in relapse multiple transfusions caused erythropoiesis to change from megaloblastic to normoblastic without other therapy. Subsequent administration of liver extract produced reticulocytosis only if the erythrocytes were less than 5 million. These results were attributed to the abolition of anemia anoxia. Because these findings were somewhat unexpected a similar study has been made and is the subject of this report.

The effects of transfusions of whole blood or packed erythrocytes sufficient to produce a normal hematocrit have been studied in four patients with untreated pernicious anemia. Bone marrow aspirations were studied before and after transfusion and after B_{12} therapy. In each patient erythropoiesis remained megaloblastic until B_{12} was given although in some instances a shift from early megaloblasts to more mature forms occurred. In every patient the absolute reticulocyte count decreased following transfusions. Subsequent administration of B_{12} produced a definite though small response in the reticulocytes. The peak of this response occurred in eight to ten days. One patient who received only 250 cc. of plasma before B_{12} had a normal reticulocyte response.

It is concluded that in these patients megaloblastic erythropoiesis was depressed by transfusions but normoblastic maturation did not occur without specific therapy.

RELATION BETWEEN THE ZONA RETICULARIS AND THE ZONA FASCICULATA OF THE ADRENAL CORTEX AND RENAL HEMODYNAMICS. *J. M. Little,* E. H. Yount and W. M. Kelsey.* Depts. of Physiology and Pharmacology, Medicine and Pediatrics, and the Laboratories of the A. H. Robins Co., Inc., Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

Inulin and PAH clearances were obtained in each of ten unanesthetized dogs on three days to establish control levels. The dogs then received daily intramuscular injections of ACTHAR-gel, 100 mg./M² for ten to twelve days, and the clearances were repeated between the fourth and twelfth day of administration. In twenty-nine determinations, each the average of three periods, the mean changes in function and the range compared to the controls were: C_{in}, +43.7 per cent (+6 per cent to +95 per cent); C_{PAH}, +52.4 per cent (−1 per cent to +117 per cent). Five dogs received rhothane (DDD) in daily oral doses of 14.5–200 mg./kg. for seven to sixty-six days. In fourteen determinations the mean changes from control and the range were: C_{in}, −21.5 per cent (+13 per cent to −44 per cent); C_{PAH}, −13.4 per cent (+16 per cent to −30 per cent). While continuing rhothane, ACTH administration was repeated. In nineteen determinations the mean changes from control and the range were: C_{in}, −3.2 per cent (+12 per cent to −19 per cent); C_{PAH}, +7.1 per cent (+42 per cent to −16 per cent). Four dogs received rhothane (200–300 mg./kg. for seven to fourteen days) and ACTH simultaneously. In ten determinations the mean changes from control and the range were: C_{in}, +16.5 per cent (+54 per cent to −5 per cent); C_{PAH}, +17.7 per cent (+39 per cent to −6 per cent). The animals remained in good health without special care. Autopsies showed no renal damage and varying degrees of adrenal atrophy confined to the inner two zones. Conclusion: Alterations of the level of function of the zonae reticularis and fasciculata affect the GFR and RPF in the dog without appreciably altering the electrolyte balance.

EFFECTS OF ACUTE ALTERATIONS IN CARBOHYDRATE METABOLISM ON THE NET SPLANCHNIC KETONE PRODUCTION IN MAN. *H. T. McPherson, E. E. Werk, F. L. Engel* and J. D. Myers.** Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.

Utilizing hepatic venous catheterization, we have previously reported a technic for studying net splanchnic ketone production (NSKP) quantitatively in man before and after the intravenous infusion of sodium octanoate, a known ketone precursor. The present study concerns the effects on NSKP of acute alterations in carbohydrate metabolism in mildly ill males with non-metabolic illnesses, studied in the postabsorptive state.

Four groups of subjects were studied: (1) the control group receiving octanoate alone; (2) those receiving glucose mixed with octanoate; (3) those receiving glucose + insulin mixed with octanoate; and (4) a group studied three hours after a brief period of hypoglycemia.

Group	Basal	NSKP μm/min./M²	During Na Octanoate
Control (octanoate alone)	34 ± 5.20	158 ± 6.86	+124 ± 7.36
Glucose in octanoate	35.8 ± 6.60	106.3 ± 22.37	+70.5 ± 18.34
Glucose + insulin in octanoate	36.6 ± 12.06	125 ± 17.14	+88.4 ± 6.07
Three hours after hypoglycemia	160.7 ± 46.0	266.3 ± 43.5	105.6 ± 27.8

In both the glucose and glucose + insulin groups there was a statistically significant reduction in the increment of NSKP expected during octanoate infusion. Three hours after a brief period of hypoglycemia there was a markedly increased basal NSKP; however octanoate infusion induced no greater rise in NSKP than that of the control groups. The relationship of these quantitative findings to the so-called "Somogyi effect" noted clinically will be discussed.

EVALUATION OF RADIOSULFATE AS A MEASURE OF EXTRACELLULAR VOLUME IN EDEMATOUS PATIENTS. *L. L. Madison, H. C. Teng, D. W. Seldin,* A. F. Reid and R. MacDonald.* Depts. of Internal Medicine and Biophysics, Southwestern Medical School of The Univ. of Texas, Dallas, Tex.

To evaluate radiosulfate as a means of measuring extracellular volume in edematous states, three approaches were planned in order to circumvent lack of adequate reference standards: the time of diffusion equilibrium was empirically determined by comparing concen-

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trations of S^{35} simultaneously in plasma, edema and ascites for fifteen hours; the validity of calculations of the S^{35} space was tested by comparing theoretic time curves of appearance of S^{35} in edema fluid with experimental curves; decrements in extracellular volume (diuresis or paracentesis) were compared with changes in radiosulfate space in the same subjects. Eleven edematous subjects were studied and eight restudied when edema-free.

Results indicate that radiosulfate is a suitable agent for measuring extracellular volume in edematous states for the following reasons: (1) mean time for diffusion equilibrium between plasma and ascitic fluid is 5.5 hours and between plasma and edema fluid is 11.3 hours; (2) mean changes in radiosulfate space from edematous state (24.8 L.) to edema-free state (11.4 L.) compared favorably with decrements in extracellular volume reflected by decreases in weight (13.6 kg.); (3) theoretic (derived from plasma data only) and experimental time curves of appearance of S^{35} into edema fluid were in excellent agreement.

SOME METABOLIC CHARACTERISTICS OF TUBERCULOUS ANIMALS. *S. P. Martin,* R. Green and C. D. Cooper.* Depts. of Medicine and Bacteriology, Duke Univ. School of Medicine, Durham, N. C.

Tissues of animals infected with Mycobacterium tuberculosis have a low succinic dehydrogenase activity and this can be restored by a nucleotide from normal tissue. The activity of this nucleotide can be duplicated by coenzyme A which has been treated with nitrous acid. This study deals with other metabolic effects noted in tuberculous animals.

Studies were carried out on the ability of infected animals to acetylate paraaminobenzoic acid. Control and infected guinea pigs were given 1 mg. of paraaminobenzoic acid and urine collected over a twelve-hour period. The free and acetylated derivatives were measured. The degree of acetylation in the control animals was 0.883 ± 0.037, in the infected animals 0.635 ± 0.075 mg. ($p < 0.02$). Studies were also carried out on non-protein sulfhydryl content of the tissues. The kidneys of infected animals have an elevated non-protein sulfhydryl content. Control values were 77 ± 3 mg. of glutathione per 100 gm. of tissue compared with 107 ± 9 ($p < 0.01$) for the infected animals.

It is suggested that these changes indicate disturbance in metabolism of high energy compounds and in protein synthesis which may account for part of the clinical picture of this disease.

MIXING IN BIOLOGICAL SYSTEMS. *G. R. Meneely.** Research Laboratory and Radioisotope Unit, Thayer V. A. Hospital, and Dept. of Medicine, Vanderbilt Univ. School of Medicine, Nashville, Tenn.

A differential equation may be derived from the assumptions that the rate of mixing or disappearance of tracer substances is not only proportional to concentration but also inversely proportional to time. The integral of this equation is:

$$\text{Exponential form: } y = C_1 t^{-k}$$
$$\text{Log form: } \log y = -k \log t + \log C_1.$$

Since the tracer is already at a finite dilution when introduced the paradox of divergence of the time integral at zero time is resolved. This formulation or one derivative from it provides extraordinarily closely fitting curves to the most diverse sorts of biological mixing data such as the disappearance of radiopotassium from the blood of rats, of radiosodium from the blood of man, of radiobromine from the blood of the dog, of diiodotyrosine from the blood of the rat, of methenamine mandelate from the blood of man, and of helium mixing in the human lung. By double logarithmic plot it is easy to test whether given data may be fitted and the constants C_1 and k may be evaluated directly.

RELATIONSHIPS BETWEEN HALF-SECOND EXPIRATORY CAPACITY TEST AND LUNG VOLUMES AND INDEXES OF INTRAPULMONARY GAS DISTRIBUTION IN EIGHTY-FIVE PATIENTS WITH VARIOUS PULMONARY DISORDERS. *W. F. Miller, R. L. Johnson, Jr. and Nancy Wu (introduced by D. W. Seldin*).* Dept. of Internal Medicine, Southwestern Medical School of The Univ. of Texas, Dallas, Tex.

By plotting in a quadrantic system pulmonary stroke volume (estimated from vital capacity) and velocity air-flow (estimated from half-second expiratory capacity, 0.5 Sec. EC), precise expressions of the nature and extent of ventilatory insufficiency are obtained. By this method ventilatory defects were characterized as: obstructive (decreased velocity air-flow), restrictive (decreased pulmonary stroke volume), and combined. To examine the significance of ventilatory disturbances revealed by this analysis the anatomic extent of disorders was estimated

from residual volumes and total capacities, while their physiologic significance was estimated from indexes of intrapulmonary mixing. The obstructive group revealed normal or increased total capacities (TC = 133 ± 33 per cent) and residual volumes (RV = 149 ± 32 per cent). The restrictive group revealed decreased to normal TC (74 ± 11 per cent), and normal to increased RV (94 ± 29 per cent). The combined group showed normal to decreased TC (87 ± 14 per cent) and decreased to increased RV (136 ± 50 per cent), depending on the extent of the obstructive component. Thus classification of ventilatory defects by 0.5 Sec. EC tests correlates well with anticipated anatomic alterations in lung volumes. The 0.5 Sec. EC correlated excellently with the index of intrapulmonary mixing (r = 0.924). Thus velocity air-flow determines in part effective ventilatory gas exchange.

The classification of ventilatory disturbances by 0.5 Sec. EC test provides excellent insight into the anatomical nature of the disturbances as well as their physiologic significance.

TREATMENT OF HYPERTENSIVE EMERGENCIES WITH PARENTERAL RESERPINE. *J. H. Moyer,* W. M. Hughes, W. C. Daeschner, Jr. and E. W. Dennis.** Baylor Univ. College of Medicine, Houston, Tex.

Three groups of hypertensive patients were treated with reserpine by parenteral administration for periods varying from one to seventeen days: (1) severe, progressive, hypertensive cardiovascular disease (ten patients with malignant hypertension and four patients with severe, benign essential hypertension); (2) toxemia of pregnancy (six patients); and (3) acute glomerulonephritis in children (eight patients). The drug was administered at intervals of four to twenty-four hours either intravenously or intramuscularly in doses of 2.5 to 5 mg. in adults and 25 to 100 μg./kg. of body weight in children. The blood pressure response after administration of reserpine was characterized by a latent period of one to three hours following which the blood pressure usually decreased to normotensive or moderately hypertensive levels for a duration of two to twenty-four hours or longer. All of the patients except one obtained a significant reduction in blood pressure and 70 per cent became normotensive in the supine position. Excessive hypotension was rarely observed. Papilledema disappeared in seven of eleven patients, and hypertensive encephalopathy was relieved in four of six patients. It is concluded that parenteral reserpine is an effective and safe agent for the therapy of hypertensive emergencies.

ROLE OF FEMALE GENITAL FUNCTION IN CREATINE METABOLISM AS REVEALED BY THE SERUM CREATINE TOLERANCE TEST. *J. H. Peters,* R. Snyderman and J. C. Crutchfield.* Investigative Medicine Service, V. A. Hospital, Atlanta, Ga.; Dept. of Medicine, Emory Univ. School of Medicine, Emory University, Atlanta, Ga.; and the Dept. of Research Medicine, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

The sex differences long-recognized in creatine metabolism have been indirectly explained by the demonstration that androgen therapy can suppress creatinuria in preadolescent boys and in disease states. However, since the female gonads might still be a factor in the maintenance of creatine intolerance in females, studies were undertaken using serum creatine tolerances. Normal females were compared with postmenopausal individuals and with patients previously subjected to ablation of ovarian and/or uterine function by surgery or radiation.

Results indicate that the relative creatine intolerance characteristic of the adult female is not affected by the presence or absence of functioning sex organs, but the findings do suggest that inability to dispose of exogenous creatine in serum increases with age independent of gonadal status. From these findings it may be concluded that androgen secretion in the male, rather than female sex function, is responsible for the well known difference in creatine metabolism.

FACTORS INFLUENCING PULMONARY COMPLIANCE IN HEART DISEASE. *W. W. Pryor, J. B. Hickam,* E. B. Page and H. O. Sieker.* Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.

In congestive heart failure the lungs become stiff (low compliance). The role this change in pulmonary distensibility plays in producing cardiac dyspnea has stimulated considerable interest. However, little has been done to define the effect of pressure changes in the various partitions of the pulmonary vasculature and parenchymal alterations on compliance. Patients with mitral stenosis, apparent primary pulmonary hypertension, pulmonary stenosis and normal subjects receiving rapid infusions of albumin were studied to find some of the answers to this problem. Compliance is measured as: lung volume change (liters)/intraesophageal

pressure change (cm. H_2O) (normal .170 ± .05 lying).

Results revealed (1) "PC" pressure need not be elevated since subjects with apparent primary pulmonary hypertension had low compliance (e.g. average .090); (2) all patients with elevated pulmonary artery pressure had reduced compliance (e.g. mitral stenosis .075, pulmonary hypertension .090); (3) subjects with pulmonary stenosis have compliance values which are high normal (e.g. .205); (4) normal subjects have a fall in compliance following rapid infusion of albumin in amount sufficient to elevate central venous pressure 6–8 mm. Hg (e.g. control .123, after albumin .087).

Conclusions: (1) pulmonary hypertension is associated with stiff lungs; (2) elevation of "PC" pressure is not necessary for this change; and (3) permanent parenchymal changes need not be present.

CLINICAL EVALUATION OF IRON TURNOVER STUDIES. *C. E. Rath, R. Peterson and P. McCurdy (introduced by H. Jeghers*).* Dept. of Medicine, Georgetown Univ. School of Medicine, Washington, D. C., and the National Inst. of Health, Bethesda, Md.

Following the studies of Huff on the rate of plasma clearance and red cell utilization of intravenously injected radioactive iron, there has been considerable dispute regarding the clinical value of such studies. The present report deals with a clinical evaluation of plasma and red cell radioiron turnover in twenty patients (twenty-eight turnovers) and a comparison of the findings with those in fifteen normal adult males and females.

The patients studied included iron deficiency, refractory anemia, anemia of Addison's disease, hemochromatosis, a variety of hereditary hemoglobin abnormalities, aplastic anemia, myeloid metaplasia, uremia, cirrhosis and acquired hemolytic anemia.

It is concluded that iron turnover rate studies, when properly interpreted, give valid information regarding marrow function and red cell destruction and that the information thus obtained is of value in selecting patients for splenectomy and in the diagnosis of certain obscure anemias. Calculations of value include the metabolic iron pool size, number of pools turned over per day, mg./Fe turned over per day, per cent and rate of incorporation of RaFe into circulating red cells, and per cent red cell mass turned over per day. Substantiating data will be presented.

BIOLOGIC DECAY RATES AND EXCRETION OF RADIOCESIUM, Cs^{134}, IN DOGS. *C. T. Ray,* G. E. Burch* and S. A. Threefoot.** Tulane Univ. School of Medicine, New Orleans, La.

Aspects of cesiokinetics were studied with Cs^{134} in three dogs observed continuously under metabolically controlled conditions for forty-seven days. Cl^{35}, Na^{23} and K^{39} were studied simultaneously. The data indicated: (1) 0.4 to 0.8 of Cs^{134} in the plasma was transferred per minute to the extraplasma compartments and 0.04 to 0.06 of that in the extraplasma compartments returned to the plasma per minute. (2) Half of the injected tracer was excreted in the urine and stools in seventeen to thirty-nine days, twenty-seven to forty-six days being required to excrete half in the urine alone. From 50 to 55 per cent of the injected dose was recovered in urine, 8 to 14 per cent in stools, 8 to 37 per cent remaining in the body. (3) At no time was equilibrium of distribution reached. (4) Rates of urinary clearance of Cs^{134} varied from 43 to 57 per cent of K^{39}. (5) Cs^{134} is not as satisfactory a tracer of K^{39} as is Rb^{86}.

CORONARY BLOOD FLOW AND MYOCARDIAL CARBOHYDRATE METABOLISM IN MITRAL STENOSIS. *J. L. Read, R. R. Porter* and S. C. Bradford.* Cardiovascular Section of the Medical Service and Cardiovascular-Pulmonary Research Laboratory, McGuire V. A. Hospital, and the Cardiovascular Div. of the Dept. of Medicine, Medical College of Virginia, Richmond, Va.

In six patients with advanced mitral stenosis, the coronary blood flow was measured using the technic of coronary sinus catheterization and the nitrous oxide method of Kety and Schmidt. Cardiac output was determined by the Fick principle. In addition, direct analyses were made of the coronary A-V differences of oxygen, glucose, lactate and pyruvate.

The coronary blood flow was reduced in all cases, with a range of 57 to 74 cc./100 gm./min. compared to the normal range of 91 to 103 cc./100 gm./min. The coronary A-V oxygen difference varied from 9.5 vol. per cent to 15.38 vol. per cent compared to the normal range of 10.00 to 12.00 vol. per cent. The cardiac index in the six cases was reduced, with a range of 1.36 to 3.93 L./min./M^2 and a mean of 2.35. The mean coronary glucose A-V difference was 5.5 mg. per cent compared to the normal of 2.66 mg. per

cent. The mean coronary lactate A-V difference was 1.93 mg. per cent and the mean coronary pyruvate A-V difference was 0.65 mg. per cent, essentially normal values.

The significantly reduced coronary blood flow indicates either a reduced requirement for coronary blood flow or an inability to maintain a normal coronary flow because of the stenotic mitral valve. Since the reduction in coronary blood flow correlated with the reduction in cardiac index, with the increase in the coronary oxygen A-V difference and with the calculated left ventricular efficiency, it would appear that the latter is the most likely explanation. Because of the reduced coronary blood flow, adaptive mechanisms apparently allow a greater extraction of glucose than normally occurs with a given arterial glucose level.

ACUTE EFFECTS OF MERCUHYDRIN ON CARDIAC OUTPUT AND URINARY EXCRETION. *A. Ruskin (introduced by R. Gregory*)*. The Univ. of Texas Medical Branch, Galveston, Tex.

Right intracardiac pressures have been found by Lenègre to decline right after mercurial administration, indicating a possible immediate improvement in cardiac output *before* its direct renal effects in heart failure.

Twelve patients were given 2 cc. of mercuhydrin intravenously. Two fifteen-minute control periods were compared to three fifteen-minute postdrug periods. In seven subjects not in heart failure urine sodium and chloride (mEq./min.) rose rapidly in all. Urine potassium rose in three, but fell later below control levels in six cases. Urinary volume rose significantly in four patients. Venous pressure varied irregularly. Cardiac output (ballistocardiographic) rose immediately in four cases (excitement?) but declined (later especially) in six, remaining unchanged in one. Five heart failure patients all showed rapid rises in sodium, then chloride and water excretion, a rise in potassium excretion in four cases and a fall in one case. Venous pressure fell rapidly in four patients, and was unchanged in one; later ascended in two. Cardiac output fell early in three cases, but rose *after* diuresis in four of five patients above control levels.

The results reaffirm the *primary* renal mechanisms of mercurial diuresis, and the *secondary* cardiac effects.

EFFECT OF HEXAMETHONIUM-INDUCED GANGLIONIC BLOCKADE ON RENAL FUNCTION IN PATIENTS WITH CONGESTIVE HEART FAILURE. *H. W. Schnaper, R. D. Kovach and E. D. Freis.** V. A. Hospital and the Georgetown Univ. Hospital, Washington, D. C.

The improvement in cardiovascular function recently reported by Kelley, Freis et al. and Brodh and coworkers following administration of ganglionic blocking agents to patients in congestive heart failure prompted the current investigation into the physiologic changes in renal function in patients in congestive failure in response to acute doses of hexamethonium. It was found in eight control studies that renal plasma flow (RPF) was diminished and glomerular filtration rate (GFR) proportionately less diminished in the subjects with congestive failure, resulting in an increased filtration fraction (FF). After intravenous dosage of hexamethonium sufficient to cause ganglionic inhibition, as measured by sustained diminution in blood pressure and mydriasis plus conjunctival injection, both GFR and RPF decreased in four cases in the first fifteen to thirty minutes, with little resultant change in FF. After this initial drop, and despite continued inhibition of the ganglionic impulses, RPF and GFR both returned to control values or above, keeping FF relatively unchanged. In four cases there was no drop in RPF and GFR after hexamethonium injection, and in two cases the rebound values exceeded the control values.

These data are at odds with those reported by Brodh and coworkers who found a significant increase in RPF in congestive failure patients following blockade with dibenzylene.

SPECIFIC THERAPY FOR SALICYLATE POISONING. *J. E. Schreiner, L. B. Berman, J. Griffin and J. Feys (introduced by L. H. Kyle*)*. Dept. of Medicine, Georgetown Univ. Hospital, Washington, D. C.

Salicylates account for 4 per cent of fatal poisonings due to solids and liquids in the United States. Surprisingly little data are available on fatal dosages, blood levels, distribution of salicylate, protein binding, etc. There has been no specific therapy. Abel in 1913 removed salicylate from animals by vividiffusion. Doolan et al. reported removal of some salicylate from a human during one hour of dialysis. Successful dialysis has not been reported.

This report concerns a patient who ingested 210 gm. of aspirin and was comatose with hyperthermia, hyperpnea, ketosis, absent corneal reflexes and a blood salicylate level of 90 mg. per

cent. Dialysis resulted in rapid clinical and chemical recovery. Hypoprothrombinemia did not become normal until forty-eight hours after the salicylate level was zero.

Preliminary calculations derived a volume of distribution for salicylate compatible with extracellular fluid. Studies of protein binding showed no interference with dialysis. The clinical course is compared with a patient who ingested a smaller amount of aspirin but had a similar blood level and was treated supportively. The amount of salicylate recovered in the bath in six hours in Case 1 exceeded that recovered in the urine in 144 hours in Case 2.

ANTIHYPERTENSIVE EFFECT OF METAL BINDING AGENTS. *H. A. Schroeder,* H. M. Perry, Jr. and E. M. Menhard.* Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.

Non-neurogenically acting antihypertensive agents (thiocyanate, nitroprusside, azide, hydrazinophthalazines, mercaptans) have in common an affinity for transition and nearby metals. Therefore, 5 mg. each of a series of nine disodium ethylenediamine tetraacetates (EDTA) of increasingly tightly bound divalent metals was assayed for vasoactivity in groups of normotensive and renal hypertensive rats, blood pressure being recorded on a Sanborn electromanometer. The significant changes occurred in hypertensive but not normotensive animals, mean diastolic pressure being depressed 30, 42, 28, 43, 60 and 50 mm. Hg by hydrogen, calcium, manganous, chromous, ferrous and cobaltous chelates, respectively, and less than 20 mm. by zinc, cupric and nickelous chelates. Vasoactivity became negligible when the log K_2 of the chelate exceeded 16.5, indicating tight binding. Similar differential effects were observed with the tetrahydrogen and lesser with the magnesium chelates while the ferric was pressor. A polyaminocarboxylic acid and 8-hydroxy quinoline were likewise depressor only in hypertensive rats. Zinc, ferrous and manganous ions as chlorides were relatively inert, nickelous was pressor and cobaltous differentially depressor. No marked effect was observed after oral or parenteral calcium or magnesium EDTA in hypertensive patients. These results are consistent with the *in vivo* binding of metal, the logs K_2 of which approximate 16.5 for EDTA.

CEREBRAL CIRCULATORY EFFECT OF THE ADMINISTRATION OF 3 PER CENT CO_2 TO SUBJECTS WITH CHRONIC PULMONARY INSUFFICIENCY. *W. Sensenbach, L. Madison and S. Eisenberg (introduced by E. S. Sulkin*).* Dept. of Internal Medicine, Southwestern Medical School of The Univ. of Texas, and the Dept. of Medicine, V. A. Hospital, Dallas, Tex.

CO_2 3 per cent, O_2 21 per cent, CO_2 3 per cent, and O_2 97 per cent were administered to fourteen subjects with chronic pulmonary insufficiency. Arterial O_2 saturation, pCO_2 and pH were determined before and after, CBF at the end of, each breathing period.

Administration of 3 per cent CO_2, 97 per cent O_2 significantly changed CBF (47 per cent increase) and CVR (25 per cent decrease); CBF, CVR were unaltered by 3 per cent CO_2, 21 per cent O_2; CMR O_2, CMR glu. did not change with either mixture. Arterial O_2 saturation increased (86–93 per cent) with 3 per cent CO_2, 21 per cent O_2 and 3 per cent CO_2, 97 per cent O_2 (82–100 per cent). pH did not change with either mixture. The most striking finding was the unexpectedly small rise in arterial pCO_2 after inhalation of the CO_2 mixtures, when compared to the marked hypercapnea produced with 100 per cent O_2.

The findings suggest that cerebral circulatory changes accompanying administration of high oxygen mixtures to subjects with chronic pulmonary insufficiency are more intimately related to changes in arterial O_2 saturation than to changes in blood pH or pCO_2. The slight rise in arterial pCO_2 despite administration of CO_2 loads suggests the existence of chemoreceptors in the lung sensitive to increased CO_2 tension of inspired air.

TREATMENT OF POSTURAL HYPOTENSION WITH A COUNTERPRESSURE GARMENT. *H. O. Sieker, J. B. Burnum, J. B. Hickam* and K. E. Penrod.* Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.

Postural hypotension may be a severe disabling illness which is difficult to treat. Experimentally, counterpressure to the lower half of the body will prevent symptoms. For this purpose a garment has been developed which consists of tailored graded-pressure nylon-elastic knit trousers that apply 15 to 20 mm. Hg pressure.

To study the hemodynamic effects of this garment, an air-filled full-pressure anti-g suit which applies a similar pressure was used on eight postural hypotensive patients. Arterial

pressure, cardiac output and pulmonary arterial pressure were determined while supine and erect, unprotected and protected. The suit alleviated the symptoms including syncope which occurred with standing. The average fall of mean arterial pressure with tilt was 45 mm. Hg unprotected and 8 mm. Hg protected. With tilt the average decrease in cardiac output was 46 per cent without counterpressure and 21 per cent with counterpressure. The mean pulmonary arterial pressure fell 6–8 mm. Hg with release of counterpressure while standing. This evidence indicates that the pressure suit is effective by increasing peripheral arterial resistance and venous return to the heart.

Individually tailored elastic garments have been used in three of the severest cases of postural hypotension for twelve to twenty-two months. The suit has been practical and successful in returning these patients to useful activity.

ELECTROMAGNETIC MEASUREMENT OF BLOOD FLOW THROUGH INTACT HUMAN ARTERIES. *M. P. Spencer, A. B. Denison, Jr., W. F. McGuire and R. T. Myers (introduced by H. D. Green*).* Depts. of Physiology and Pharmacology, Urology and Surgery, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C

Measurements of blood flow through intact blood vessels has been made practical by a recent development of the electromagnetic method of flow recording. The sources of instability of previous systems have been considerably reduced by use of a square-wave magnetic field and an AC amplifier containing a chopping circuit to eliminate electrode voltages induced during reversal of the magnetic field.

The unit applied to the blood vessel consists of a small U-shaped electromagnet imbedded in plastic with a slot at the open end to accommodate the vessel and provided with two stainless steel electrodes. The size and shape of this magnet electrode assembly is altered to meet the particular application. The vessel unit can be sterilized and is easily applied. The sensitivity of each unit, determined by *in vitro* calibrations of blood forced through an artery or vein, is linear and constant.

To date the meter has been applied to the renal artery in four instances of nephrectomy and to the femoral artery during replacement of a thrombosed segment with a vein graft. These and other applications will be discussed.

THE ANEMIA OF UREMIA: HEMOLYTIC STATE MEASURED BY THE RADIOCHROMIUM METHOD. *D. A. Sutherland, S. McCall, F. Jones and E. E. Muirhead.** Radioisotope Unit, Dallas V. A. Hospital, and the Depts. of Medicine and Pathology, The Univ. of Texas Southwestern Medical School, Dallas, Tex.

The anemia of chronic uremia was studied in eleven patients by means of the autotransfusion of CR^{51} tagged RBC. Two patients revealed normal RBC survival indicating a bone marrow dysfunction only. The RBC survival was significantly lowered in the remaining nine patients as indicated by the following values (in days) for the mean life-span: twenty-five, thirty, thirty, thirty, thirty-five, forty, forty-five and fifty-five (normal values eighty to ninety days). In two cases the same shortened life-span obtained by autotransfusion was detected by transfusion of tagged RBC from the uremic patients into normal subjects. The transfusion of normal RBC into one of these patients yielded a normal life-span.

Four normal dogs were subjected to bilateral nephrectomy and no dialysing procedure. Four days later the RBC from the uremic animals were tagged with Cr^{51} and transfused into normal dogs. The mean life-span of these RBC was fifteen days (normal eighty to ninety days).

The anemia of uremia in most of these examples resulted in part from a hemolytic state due apparently to alterations of the RBC. Indications for a bone marrow dysfunction were also noted.

ACUTE EFFECT OF HEPARIN ON CEREBRAL BLOOD FLOW AND CEREBRAL METABOLISM. *R. W. Talley (introduced by P. L. Day*).* Dept. of Medicine, Univ. of Arkansas School of Medicine, Little Rock, Ark.

Studies of the cerebral blood flow (CBF), cerebral oxygen utilization ($CMRO_2$), cerebral vascular resistance (CVR), and cerebral glucose utilization (CMRG) were made before and after a large dose of heparin in eleven aged patients. All of these patients were in a state hospital for nervous and mental disease and had varying degrees of mental deterioration apparently due to cerebral arteriosclerosis.

There was no correlation between degree of mental deterioration and the rate of CBF. Forty-eight to seventy-two hours after the control study the patient was given 200 mg. of heparin intravenously. Three hours after the

administration of the heparin a repeat cerebral blood flow study was made. The studies before and after the administration of heparin were compared and no statistical difference in the CBF, $CMRO_2$, CVR and CMRG was found. The average values of the CBF, $CMRO_2$, CVR and CMRG before and after heparin were as follows:

	CBF	$CMRO_2$	CVR	CMRG
Before	33.8	2.2	3.4	4.5
After	33.1	2.0	3.4	4.2

INFLUENCE OF THIOURACIL AND METHIMAZOLE ("TAPAZOLE") ON THYROID FUNCTION IN DOGS WITH SPECIAL REFERENCE TO SERUM CHOLESTEROL CHANGES. *S. Y. Tsai, L. G. May, V. A. Stembridge and R. Gregory** (with the technical assistance of A. Bennett, M. Kobayashi, M. Finley and J. R. Bateman). John Sealy Memorial Laboratory and Dept. of Internal Medicine and Pathology, Univ. of Texas Medical Branch, Galveston, Tex.

It has been suggested that the hypercholesterolemia in dogs receiving thiouracil may be due to a specific effect of the drug on serum cholesterol rather than to its antithyroid action. The absence of hypercholesterolemic effect of methimazole when given in a single daily dose would seem to support this hypothesis. The present study is designed to clarify these points.

Fifteen healthy mongrel dogs were divided into three groups: (1) no medication, (2) thiouracil in a single daily dose for two to three weeks and (3) methimazole five doses per day for three weeks. Serum cholesterols, PBI, histologic changes and thyroid I-131 uptakes were studied. Hypercholesterolemia and depression of thyroid I-131 uptake was observed in groups 2 and 3 but not in group 1. Serum PBI was found to normally low (Barker's method) and showed no significant change after the administration of thiouracil or methimazole. Histologic studies are in progress.

As hypercholesterolemia induced by thouracil and methimazole (in daily divided doses) correlates with decreased thyroid I-131 uptake, it is concluded that thiouracil does not have a specific effect on serum cholesterol and that hypercholesterolemia is a manifestation of induced hypothyroidism. The significance of normally low PBI in dogs will be discussed.

THE PERMEABILITY OF CAPILLARIES TO LARGE MOLECULES. *K. Wasserman and H. S. Mayerson.** Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.

This study of capillary permeability to large molecules was stimulated by observations of transudation from the circulation of infused plasma and normal saline solutions containing relatively large molecular weight solutes. We have followed the rates of disappearance of large molecules from plasma and their appearance and concentration in thoracic duct lymph. The leakage of larger molecules from plasma and their appearance in lymph is inversely related to their molecular weight when plasma volume and rate of plasma transudation are constant. Even relatively large molecules (dextran—average M. W. = 412,000) get into lymph, albeit in small amounts. The plasma-lymph gradients are influenced markedly by the size of the infusion volume. When the ratio of infusion to plasma volume is large, the plasma-lymph concentration gradient of all large molecules in circulation falls and the lymph flow increases regardless of the type of colloidal solution involved. This indicates that the permeability of the capillary wall to albumin, globulin and all large molecules is increased. The decreased gradient is the result not only of a fall in plasma but a rise in lymph solute concentration. These results are interpreted in terms of the pore theory as signifying a stretching of the capillary wall pores consequent to high capillary pressure from the increased volume in the circulation.

Case Reports

Retroperitoneal Pneumography in the Diagnosis of Retroperitoneal Lymphomatous Neoplasms*

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THE diagnosis and demonstration of retroperitoneal lymphomatous neoplasms is a serious and difficult problem. In most such cases physical examination, laboratory tests and conventional roentgenologic studies are unrevealing.

We have found retroperitoneal pneumography induced by the presacral insufflation of air to be a useful adjunct in the demonstration of retroperitoneal Hodgkin's disease and leukemic nodes when other diagnostic methods have failed. This procedure has provided valuable diagnostic and prognostic information with a minimum of discomfort and risk to the patient and has been of value in selection of the type of therapy.

TECHNIC

The technic of presacral air injection is not difficult. With the patient in the knee-chest position, the skin just distal and to one side of the tip of the coccyx is cleaned and infiltrated with a local anesthetic. A 3 inch, 16 gauge needle is directed obliquely upward, inward and forward under the coccyx in such a manner that its point comes to rest in the presacral, retrorectal fatty areolar tissue. An examining finger is kept in the rectum to insure proper position of the needle. The usual technic has been modified by the use of an 18 inch length of No. 50 polyethylene tubing which is threaded through the needle. The latter is removed and the tube strapped to the patient. Using a 50 cc. syringe, three-way stopcock and an adaptor fitted to the tubing, air is injected under fluoroscopic guidance. The polyethylene tubing makes it possible to give the injection conveniently and facilitates positioning of the patient so that an adequate amount of air is delivered in the desired location. About 1,000 cc. has been required for the average patient.

Routine stereoscopic frontal and single lateral roentgenograms are obtained as soon as possible after the injection. In addition, body section films of the upper and lower retroperitoneal areas are taken. When involvement of one side in particular is suspected, better delineation may be obtained if the roentgenograms are repeated after the patient has rested on the opposite side for twenty to thirty minutes. Some degree of mediastinal emphysema is present in some patients after several hours in the upright position, and in several cases this has been sufficient to obtain satisfactory mediastinal pneumograms.

No complications have been noted in a series of twenty patients studied in this manner. Transient abdominal and subdiaphragmatic discomfort has been present in most patients. Air embolism is apparently rare, only one such catastrophe (and that non-fatal) having been reported.[1] Mosca reviewed 1,500 cases in which retroperitoneal pneumography was performed without serious complication.[2] Because of its rapid absorption and probable greater margin of safety, oxygen has been used by most operators. Air has the advantage of being more conveniently administered and with its use there is a better possibility of outlining the mediastinum.

* From the Departments of Radiology and Medicine, Veterans Administration Hospital, Philadelphia, Pa.

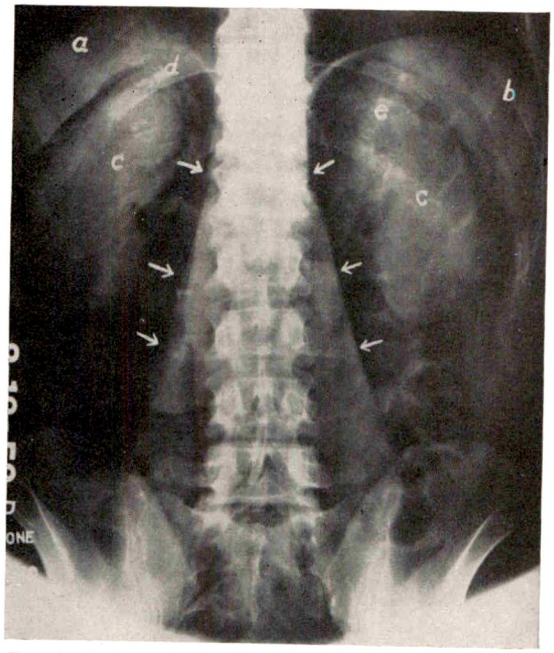


FIG. 1. Anteroposterior Bucky film of normal retroperitoneal pneumogram: *a*, liver; *b*, spleen; *c*, kidneys; *d*, right adrenal gland; *e*, left adrenal gland. Arrows indicate appearance of the normal psoas musculature and midline retroperitoneal structures.

Figure 1 demonstrates a normal retroperitoneal pneumogram. In addition to excellent delineation of the kidneys, adrenal glands, liver and spleen, the normal width and general appearance of the psoas muscles and midline retroperitoneal area is demonstrated.

The following typical cases are presented in which retroperitoneal pneumography was of value in the demonstration of retroperitoneal lymphomas.

CASE REPORTS

CASE I. J. R., a thirty year old white man, had had Hodgkin's disease since 1949, manifested by recurrences of cervical lymph node enlargement. He had been treated by both x-ray and nitrogen mustard with temporary remissions. His last course of therapy had been given eight months prior to admission, following which he had been well for about seven months. One month prior to entry persistent backache and malaise developed and on June 26, 1953, he was admitted to the Veterans Administration Hospital in Philadelphia. Physical examination, laboratory tests and routine roentgenographic studies, including an intravenous urogram, were unrevealing. Retroperitoneal pneumography (Fig. 2) revealed a large midline mass. X-ray therapy to the back gave prompt symptomatic improvement.

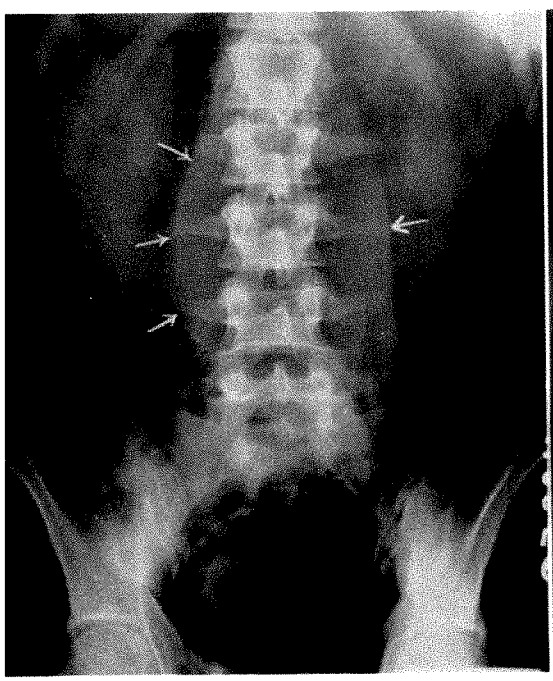
CASE II. E. B., a forty-two year old Negro man, had had chronic lymphatic leukemia since 1950. Therapy had consisted of local x-ray, nitrogen mustard and triethylene melamine. He was admitted to the Veterans Administration Hospital on October 5, 1953, with a one-month history of fatigue, leg cramps and abdominal swelling.

Physical examination revealed generalized lymphadenopathy and a moderate degree of ascites. The white blood cell count was 33,000 per cu. mm., of which 80 per cent were mature lymphocytes, 5 per cent lymphoblasts, 10 per cent smudge cells and 5 per cent polymorphonuclear leukocytes. The remainder of the laboratory studies were negative except for a moderate anemia. Roentgenograms of the chest revealed bilateral hilar lymphadenopathy. Gastrointestinal x-rays and an intravenous urogram were negative. The routine abdominal roentgenogram and stereoscopic frontal views of the abdomen after retroperitoneal pneumography were interpreted as being within normal limits. Laminagrams of the lower retroperitoneal area, however, revealed significant nodal enlargement. (Fig. 3.) Whole body x-ray therapy produced dramatic improvement in all respects and the patient was asymptomatic and had a normal hemogram when last seen in July, 1954.

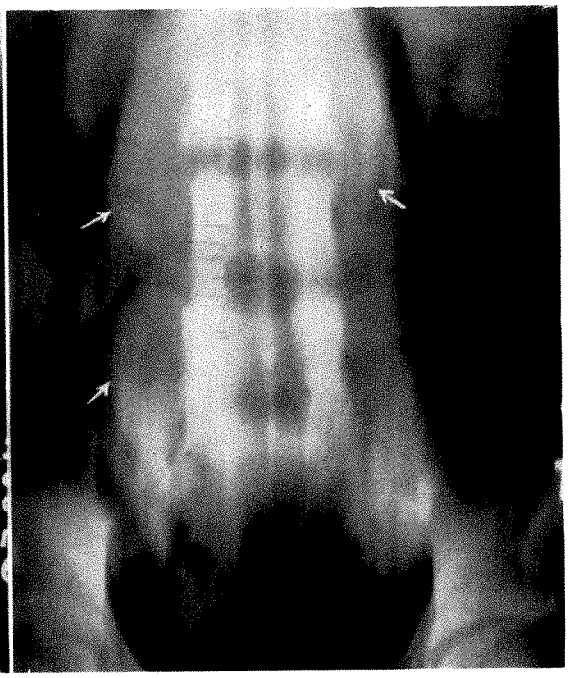
CASE III. J. D., a twenty-eight year old white man, had had Hodgkin's disease since 1952, manifested initially by cervical lymphadenopathy. Therapy with nitrogen mustard and x-ray had produced remissions.

In February, 1954, the patient was admitted to the Veterans Administration Hospital in Philadelphia with complaints of pain in the lumbar region, fever and malaise. Retroperitoneal involvement was suspected but physical examination, laboratory tests, conventional roentgenograms and retroperitoneal pneumography were negative. Symptoms persisted and a repeat presacral air study in July, 1954, revealed a definite mass. (Fig. 4.) Localized x-ray therapy is contemplated.

CASE IV. J. C., a forty-one year old white man, had had Hodgkin's disease since 1951, initially manifested by cervical lymphadenopathy. Therapy had consisted of x-ray therapy and nitrogen mustard. The patient had been well for a period of two and a half years, follow-



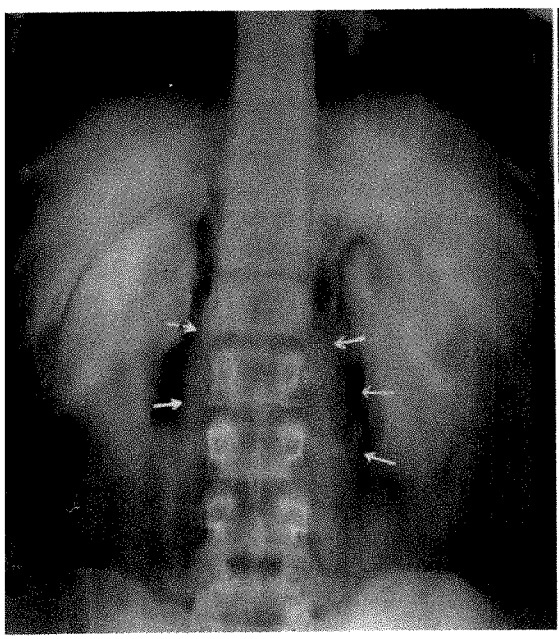
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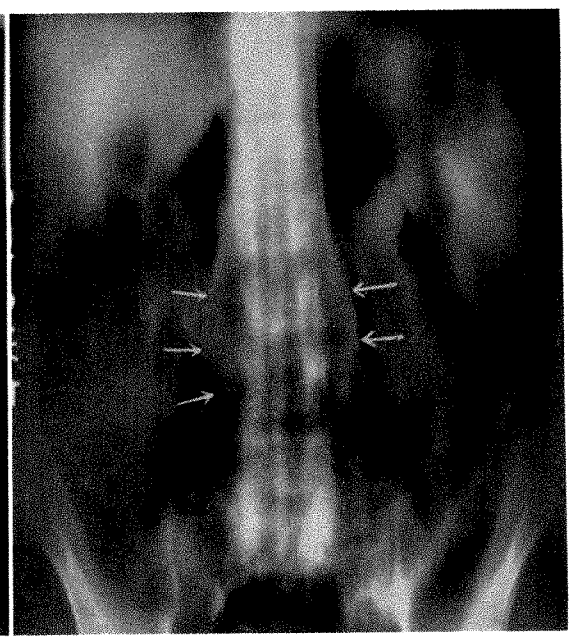
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FIG. 2. Case I. J. R. Hodgkin's disease. Anteroposterior Bucky film in which arrows indicate large retroperitoneal mass.

FIG. 3. Case II. E. B. Chronic lymphatic leukemia. Laminographic study of lower midline retroperitoneal area following pneumography. Arrows indicate enlarged nodal masses.



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FIG. 4. Case III, J. D. Hodgkin's disease. Laminographic study following retroperitoneal pneumography showing midline retroperitoneal mass.

FIG. 5. Case IV, J. C. Hodgkin's disease. Laminographic study following retroperitoneal pneumography. Arrows indicate retroperitoneal mass.

ing which weakness, cough, dyspnea and pain in the back developed. He was admitted to the Veterans Administration Hospital in Philadelphia with these complaints on August 13, 1954. Physical examination at this time revealed pallor, malnutrition, generalized lymphadenopathy and splenomegaly. Laboratory studies were not remarkable except for a moderate anemia. Roentgenogram of the chest revealed large mediastinal nodes. Other roentgenographic studies, including an intravenous urogram, were normal. Body section films after retroperitoneal pneumography revealed a midline retroperitoneal mass in the lumbar region. (Fig. 5.) The patient is being treated with nitrogen mustard.

Air has been utilized in roentgenology for many years as a means of contrast to differentiate tissues and organs situated in media of homogeneous density. In 1921, Carelli[3] introduced air into the renal fascia to outline the kidneys and adrenals. Following the work of Ruiz Rivas[4,5] the presacral technic for production of retroperitoneal pneumography gradually supplanted perirenal insufflation. Numerous articles have been published attesting to the value of this procedure for the demonstration of abnormalities of the kidneys and adrenal glands.

Retroperitoneal pneumography is well suited for the demonstration of midline retroperitoneal neoplastic masses. It must be emphasized that a negative study does not rule out the presence of pathologic changes. Recent experience with its use in the demonstration of metastatic nodes from testicular tumors would seem to indicate that retroperitoneal pneumography will not satisfactorily demonstrate lesions less than 4 cm. in diameter.[6]

SUMMARY

Retroperitoneal pneumography is a valuable adjunct in the diagnosis and demonstration of retroperitoneal lymphomatous neoplasms.

Modification of the usual technic by the use of a polyethylene catheter for administration of the gas is recommended. Fluoroscopy and body section roentgenography are believed to be important additions to the usual roentgenographic technic.

Four typical cases are presented in which retroperitoneal pneumography was helpful in the demonstration of retroperitoneal lymphomatous masses.

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Rupture of Aortic Aneurysm into the Pulmonary Artery*

Report of a Case Proved by Cardiac Catheterization

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NUMEROUS cases of rupture of an aortic aneurysm into the pulmonary artery have been reported in the literature during the past decade or so, and the clinical picture produced by this event has been adequately described so that at least a presumptive antemortem diagnosis can be made in many cases.[1–6] There is, however, only one report in which the diagnosis was proved by cardiac catheterization.[2] The purpose of this report is to add another such case and discuss the diagnosis and pathophysiology of this condition.

Up to the present, eighty-five cases of aortic aneurysm with rupture into the pulmonary artery have been reported.[1–6] Only seven were diagnosed before death. During the past year two cases were diagnosed at this hospital; one antemorten (case reported herein), the other postmortem.

The great majority of aneurysms of the thoracic aorta are in the ascending portion, and in view of the intimate relationship of the pulmonary artery to the ascending aorta one wonders why there is not a greater incidence of rupture into this vessel.

The onset of this disorder may be very acute, with extreme dyspnea, cough and precordial pain. Rarely the patient is relatively asymptomatic, complaining of mild to moderate dyspnea. The subsequent course is probably dependent in part on the size of the fistula. The patient may have progressive signs of right heart failure. The pulse pressure is wide and other peripheral arterial signs typical of aortic insufficiency are present. A systolic or continuous thrill is felt over the pulmonic area, and a continuous machinery-like murmur with systolic accentuation is heard over the same area. X-ray of the chest usually reveals evidence of aneurysm of the thoracic aorta and occasionally a prominent pulmonary conus, the latter sometimes being obscured by the aneurysm. The electrocardiogram usually reveals right axis deviation or right ventricular hypertrophy.

Patients with this disorder may die suddenly with the onset of the rupture or live for days or months, the average duration of life being three to four weeks. The longest survival time recorded is four years, reported by Clerc.[7] This, however, is very unusual, and there is some question as to the time of onset in this case.

This condition is most frequently misdiagnosed as aortic insufficiency but several points may be kept in mind to guard against this error. The continuous character of the murmur is in marked contrast to the to-and-fro murmur of aortic insufficiency. In the electrocardiogram right axis deviation is the rule, while in aortic insufficiency left axis deviation with left ventricular hypertrophy is characteristic. The peripheral arterial signs are the same in both conditions. Increased pulsation of the pulmonary artery is frequently seen in cases of aortic-pulmonary fistula whereas in cases with aortic insufficiency there is prominent pulsation of the aorta. The aortic second sound is present in the former but tends to be absent in the latter condition.

Patent ductus arteriosus enters into the differential diagnosis. A negative past history of cardiac symptoms or signs is helpful. The onset is not nearly as abrupt or drastic in patent ductus as in the acquired communication, and positive serologic reactions for syphilis and evidence of aortic aneurysm favor the diagnosis of acquired aortic-pulmonary fistula. The machinery-like murmur is characteristic of both conditions.

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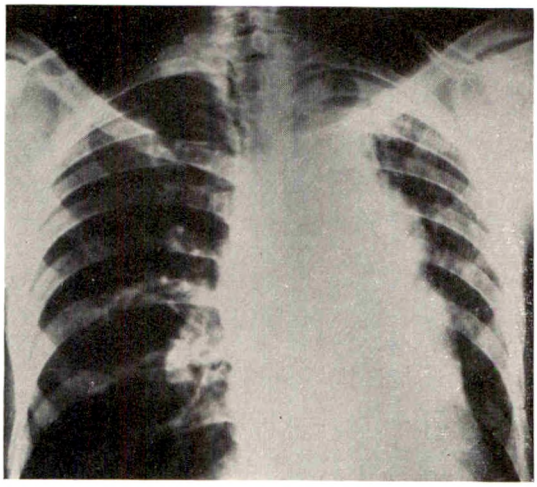


FIG. 1. Dilatation of the aortic arch, prominence of the pulmonary artery and moderate left ventricular enlargement.

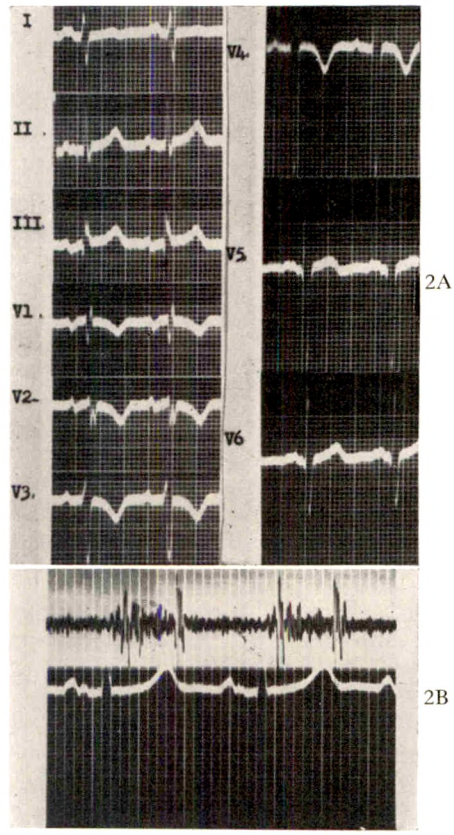


FIG. 2. A, note the right axis deviation. B, the phonocardiogram shows a continuous murmur with a systolic accentuation.

Rupture of the sinus of Valsalva into the pulmonary artery, right ventricle or right auricle may give signs and symptoms practically identical with those of aortic aneurysm with rupture into the pulmonary artery.[8–10] Absence of radiologic aortic aneurysm and negative serologic tests for syphilis favor the diagnosis of rupture of the sinus of Valsalva.

Why is there evidence of right heart strain in patients with this condition, while in patients with patent ductus arteriosus left heart strain is evident? This is probably due to long-standing pressure of the aneurysmal sac on the pulmonary artery prior to rupture, with resultant increased resistance distal to the pulmonary valve and right ventricular strain.[1,2] Another theory as regards right heart strain is based on the direction of blood flow from the shunt.[1] If the jet of blood is directed away from the pulmonic valve, there is little increased resistance to right ventricular outflow; however, if the jet is directed at a right angle to the pulmonary artery or towards the pulmonic valve, a definite increase in resistance to outflow occurs.

CASE REPORT

F. B., a fifty-two year old Negro dock laborer, was admitted to Charity Hospital on November 13, 1952, with a chief complaint of dyspnea of ten days' duration. He stated that about one month before admission he was struck on the right shoulder by a loading machine. At this time he noticed some aching but the skin was not broken. The next day he noticed a small protrusion above the right clavicle which would appear when he lay down and disappear when he sat up. He attributed this protrusion to the injury but paid no particular attention to it since it caused him no discomfort. About ten days before admission he began to notice severe weakness, dyspnea, cough and swelling of the feet. The dyspnea became progressively worse, and the cough productive of a white, foamy sputum. Hoarseness also developed.

There appeared to be no pertinent data in the past or family history.

Physical examination revealed a moderately dyspneic, middle-aged colored man who did not appear acutely ill. Blood pressure was 170/90, equal in both arms, pulse was 104 and regular, respiration rate 20. Pertinent abnormalities included the following: Above the right clavicle there was a soft, round mass which was easily reducible by pressure and which increased in size on coughing. This was believed to be a pleural hernia related to the injury of the previous month. The apex beat was in the

seventh interspace outside the anterior axillary line, forcible and diffuse. There was dullness to the left of the sternum in the second and third interspaces, and a systolic thrill was present in this region. A loud, harsh, continuous murmur with systolic accentuation was audible over the left upper chest, loudest in the second left interspace near the sternum. There were no rales in the lungs. The liver was palpable five fingerbreadths below the rib margin and somewhat tender. The spleen was not palpable. There was moderate edema of the feet and ankles. Blood picture and urinalysis were normal. Complement fixation test for syphilis was positive, the quantitative Kahn test was 8 units. Venous pressure in the right arm was 130 mm. of water, in the left 160.

Fluoroscopy revealed what was interpreted as dilatation of the transverse and ascending aorta with opacity of the aortic window. The pulmonary artery was enlarged and its pulsations increased. There was moderate enlargement of the left ventricle, and it appeared also that the right atrium was somewhat dilated. (Fig. 1.)

The electrocardiogram (Fig. 2) showed prominent S waves in leads I and V_6 and a high R in V_1. P waves were notched and T waves inverted in $V_{1, 2, 3, 4}$, diphasic in V_5. The electrocardiogram was believed to indicate right ventricular hypertrophy.

The tentative diagnosis on the basis of the initial study was syphilitic aneurysm of the ascending aorta with erosion into the pulmonary artery.

The symptoms of congestive failure responded readily to rest, digitalization, mercurial diuretics and a low salt diet. After subsidence of the heart failure an intravenous angiocardiogram was performed but satisfactory visualization of the aorta was not obtained. Cardiac catheterization (Fig. 3) revealed right ventricular pressure of 63/5 mm. Hg with the O_2 content essentially the same as that in the superior vena cava. As the catheter passed into the main pulmonary artery the oxygen content rose 6 vol. per cent. The pressure in this vessel was 63/8. Mean pulmonary capillary pressure was 26. Arterial oxygen saturation was 95 per cent and reached 100 per cent with inhalation of 100 per cent oxygen. Left ventricular output was calculated at 3.4 L. per minute and it was estimated that the pulmonary flow was about three times the systemic flow.

Following the study the patient was discharged and followed in the Outpatient Clinic. After nineteen months he remained in a fair state of health.

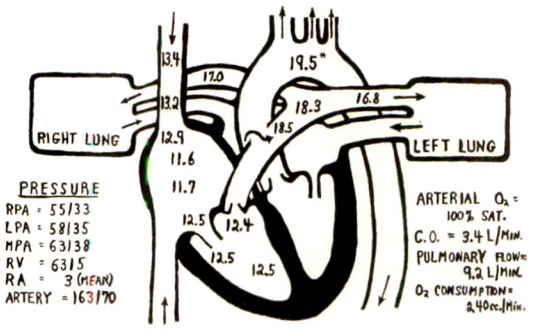


FIG. 3. Cardiac catherization results. Note the high right ventricular pressure and the 6 volume per cent elevation in O_2 content of the pulmonary artery as compared to that of the right ventricle. Asterisk denotes sample from left brachial artery.

COMMENTS

Although the subjective symptoms of this patient merely indicated congestive heart failure of fairly rapid onset, the physical signs were strongly suggestive of a communication between the aorta and the pulmonary artery, either a patent ductus or rupture of an aneurysm into the pulmonary artery. The findings on cardiac catheterization proved the presence of such a communication, and indicated that the fistula was of moderate size. The positive serologic tests for syphilis, the x-ray evidence of dilatation of the aorta and the rather sudden onset of symptoms made it fairly certain that the communication between the aorta and pulmonary artery was not a patent ductus but rather a fistulous connection with an aneurysm of the aorta.

SUMMARY

A case is reported in which erosion of an aneurysm of the aorta into the pulmonary artery, with production of a fistula between these two vessels, was proved by cardiac catheterization.

The patient was alive and in a fair state of health nineteen months after the episode occurred.

The diagnosis and pathophysiology of this condition is discussed.

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The American Journal of Medicine

VOL. XIX AUGUST, 1955 No. 2

Editorial

Clinical Usefulness of Fructose

THE recent advances which have been made in the understanding of the metabolic processes concerned with utilization of the three hexoses mainly involved in mammalian energy metabolism, glucose, fructose and galactose, have led to renewed interest in the possible clinical usefulness of sugars other than glucose. The rediscovery of identical fructose tolerance curves in normal and diabetic subjects has more particularly directed this interest toward fructose. The potential clinical usefulness of this hexose is intimately connected with a proper understanding of the metabolic transformations involved, of the rate at which they proceed and of the differences which exist between individual tissues in these respects. A brief review of present knowledge may thus be indicated.

Metabolism of Fructose in Normal Tissues. The metabolism of fructose is schematized in Figure 1. It should be immediately pointed out that the scheme is a composite one based on studies of several tissues in several species. The reactions which are part of the usual pathways of glycolysis are indicated by heavier arrows. It is clear that whenever fructose metabolism leads to the formation of an intermediate of glycolysis, further metabolism of fructose becomes identical with that of glucose. Five reactions peculiar to fructose have been described and have been numbered in the scheme. Reference will be made later to the reactions in this scheme when individual tissues are considered.

Reaction 1: Fructose can be phosphorylated in position 6 in the presence of hexokinase and ATP. The same enzyme catalyzes the phosphorylation of glucose and mannose in the same position but its affinity is different for each of these hexoses. Competitive inhibition between these substrates has been observed. The best studied mammalian hexokinase, brain hexokinase, has a greater affinity for glucose than for fructose;[1] glucose greatly inhibits the phosphorylation of fructose by this enzyme. (Table 1.) This example alone should suffice to demonstrate the danger of drawing general conclusions from observations on the capacity of an isolated tissue to metabolize a substrate which is offered alone, without naturally present competitors.

Reaction 2: Independent investigations in St. Louis,[2] Louvain[3] and Zurich[4] have established that, in liver and muscle, fructose can be phosphorylated in position 1 by a specific fructokinase. No competitive inhibition between glucose and fructose can be demonstrated for this enzyme. Since the product of this reaction, fructose-1-phosphate, is not an intermediate of glycolysis, it must undergo further modifications before entering the standard pathways of intermediary metabolism. Direct conversion of fructose-1-phosphate to fructose-6-phosphate does not appear to occur to any significant degree.[5]

Reaction 3: Fructose-1-phosphate can be split by an enzyme closely related to aldolase[5,6] to two three-carbon fragments, of which one is phosphorylated (dihydroxyacetone phosphate) and one is not (glyceraldehyde).

Reaction 4: Glyceraldehyde can be phosphorylated by a triokinase[5] to phosphoglyceraldehyde, and the two three-carbon fragments resulting from fructose-1-phosphate are now identical with those resulting from the splitting of fructose-1-6-diphosphate by aldolase; from

this point, therefore, glucose and fructose follow the same metabolic pathways up or down the chain of glycolytic reactions.

Reaction 5: Fructose-1-phosphate can also be phosphorylated in position 6 to fructose-1-6-diphosphate.[1] Whether the enzyme involved is identical with the phosphofructokinase which catalyzes the phosphorylation of fructose-6-phosphate, is not as yet established.

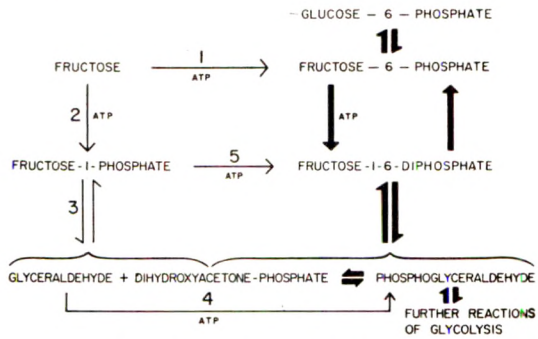


FIG. 1. Composite scheme of fructose metabolism.

When considering the metabolism of a substance to be administered to intact organisms it is important to consider not only the transformations which this substance may undergo but also the rate at which these reactions can proceed, both in the organism as a whole and particularly in each individual tissue. We shall now consider the information available on the metabolism of fructose by various tissues.

The liver is quantitatively the main site of fructose metabolism. It takes up fructose from the blood stream or from the incubation medium at a rate considerably greater than is true for glucose.[7–12] Once taken into the liver cell it is metabolized mainly by Reactions 2, 3 and 4. (Fig. 1.) The conversion of fructose to lactate, CO_2, glycogen or fatty acids exceeds that of glucose.[7,12–15] A varying portion of the fructose metabolized is transformed to glucose-6-phosphate and, by glucose-6-phosphatase action, to glucose which appears in hepatic vein blood or in the medium surrounding liver slices.[10–12] That the fructose thus rapidly metabolized yields useful energy is well exemplified by the observation of Helmreich et al.[16] who found that the capacity of the liver to produce activated two-carbon fragments capable of acetylating sulfonamide is greatly enhanced in the presence of fructose.

On the basis of data obtained by catheterization of the hepatic vein during fructose administration, Weichselbaum has estimated that up to 50 per cent of an administered fructose load is metabolized in the liver within one hour.[10] The total figure is probably considerably greater[17] since much of the fructose which had diffused into the extracellular (and probably intracellular) water later diffuses back into the blood stream to be metabolized in the liver. The ability to transform fructose to glucose is probably shared, although to a much smaller degree, by intestinal mucosa[18] and by renal tubular cells.[19]

TABLE I
MUTUAL INHIBITION OF GLUCOSE AND FRUCTOSE PHOSPHORYLATION BY HEXOKINASE OF BRAIN[1]

Incubation Period (min.)	Initial Concentration M × 10^{-3}		Fructose Used γ	Glucose Used γ	Per cent Inhibition	
	Fructose	Glucose			Fructose	Glucose
20	2.11	0	520	...	...	..
20	0	2.38	...	375	...	..
20	2.11	2.38	0	360	100	4

While brain slices or homogenates metabolize fructose at satisfactory rates, it is doubtful whether any fructose utilization by brain occurs under physiologic conditions. This is adequately explained by the presence in the intact organism of glucose concentrations sufficient to inhibit the phosphorylation of fructose by the hexokinase of brain, as previously illustrated in Table 1. A specific fructokinase has not been found in brain. That fructose does not prevent hypoglycemic symptoms in the dog was clearly shown in 1931 by Bollman and Mann.[20] They demonstrated that in the eviscerated dog blood levels of fructose of 100 mg. per cent or more did not prevent the appearance of convulsions whenever the blood glucose level was allowed to drop below a critical level.

Whether muscle can utilize significant amounts of fructose is a matter of controversy. At best, over-all fructose utilization by muscle is slow,[21–23] proceeding at one half or less the rate of glucose utilization. Possibly it is practically non-existent,[24] as suggested by Wick who found that the rate of formation of $C^{14}O_2$ from C^{14}-labelled fructose was less than 5 per cent of that from C^{14}-labelled glucose in the eviscerated nephrectomized rabbit. Hers[17] has recently reviewed the available data, added a number of

new observations, and concluded therefrom that in the intact rat most of the fructose administered reaches striated muscle after previous conversion by the liver to glucose or to other metabolites. What fructose is directly incorporated appears mainly to follow Reaction 1. (Fig. 1.) The concept of adequate fructose utilization by muscle is based largely on measurements of fructose "uptake" by isolated tissues or of peripheral arteriovenous differences; it has been further fostered by demonstration of a specific fructokinase (Reaction 2, Fig. 1) in muscle homogenates.[1] However, studies measuring not only the uptake of fructose but also its ultimate fate in muscle tissue are more meaningful, and the situation prevailing in the intact organism, including competition with glucose, should whenever possible be preserved.

TABLE II
COMPARATIVE UTILIZATION OF C^{14}-GLUCOSE AND C^{14}-FRUCTOSE BY RAT DIAPHRAGM*

Experiment	A Glycogen Synthesized from Glucose	B Glycogen Synthesized from Fructose	Ratio $\frac{A}{B}$	C Glucose Oxidized to CO_2	D Fructose Oxidized to CO_2	Ratio $\frac{C}{D}$
	μM/gm. of tissue/90′			μM/gm. of tissue/90′		
1	7.32	1.10	6.7	4.97	1.36	3.6
2	6.64	1.13	5.9	4.22	1.20	3.5

* Both hexoses are simultaneously present in equimolar concentration (20 mM/L.) Unpublished results. Experimental conditions identical with those previously described for liver[12] and diaphragm.[37]

The incorporation of fructose and glucose carbon into glycogen and CO_2 by rat diaphragm has been compared in parallel experiments, both hexoses being present in all flasks, but only one of the two having been labeled with C^{14} in each. Results are shown in Table II and clearly indicate that in the presence of equal concentrations of glucose and fructose rat diaphragm metabolizes six to seven times more glucose than fructose in the formation of glycogen and approximately three and one-half times more glucose than fructose in the conversion to CO_2. It seems reasonable to conclude that, at best, fructose utilization by muscle is small when compared with that of glucose. Red blood cells have been shown to utilize fructose only in the absence of glucose.[22] No adequate information concerning adipose tissue has become available as yet.

Even a brief review of fructose metabolism cannot be concluded without recalling the intriguing situation present in placenta and in male accessory sexual glands.[25,26] In most species at least the early placenta and one or the other male accessory sexual gland are able to convert glucose to fructose and to secrete fructose into the embryonic blood stream or seminal fluid, respectively. Why this unique situation should have been preserved throughout evolution is at present a mystery.

Metabolism of Fructose by Abnormal Tissues. Since fructose is mainly metabolized by the liver it is not surprising that the rare anomalies of fructose utilization which have been described have been found in hepatic disease. Fructose tolerance is decreased in extensive hepatocellular damage.[27] However, more recent studies[8,15,28] using intravenous fructose tolerance tests suggest that this is a late manifestation of hepatic dysfunction and that the system responsible for hepatic fructose utilization is a resistant one. Fructosuria, an entirely benign inborn error of metabolism associated with a marked decrease in fructose tolerance,[29] quite likely results from deficient hepatic fructokinase activity, although this has not been conclusively established. The metabolism of fructose in diabetes mellitus has been repeatedly studied. It has been postulated, since Minkowski's demonstration of glycogen formation from fructose in pancreatectomized dogs,[30] that diabetic tissues utilize fructose at nearly a normal rate. This has since been further substantiated both in the intact organism[9,15,31] and in isolated liver tissue;[12,14] it indicates that at least the Reactions 2, 3 and 4 (Fig. 1) are not dependent on the presence of insulin. The small portion of fructose, however, which is metabolized by way of hexokinase (Reaction 1, Fig. 1) may be influenced by insulin.[17,32] That the latter observation is of little quantitative significance is suggested by the well established normal fructose tolerance in the diabetic organism. On the other hand, it should be clearly understood that the presence of normal fructose tolerance in diabetes suggests no more than that the diabetic tissues utilize fructose as well or, for certain tissues, as poorly as normal tissues. It evidently does not justify the assumption that tissues such as brain or muscle, which utilize little or no fructose in the normal situa-

tion, utilize fructose any better in the presence of diabetes.

POTENTIAL CLINICAL USEFULNESS

It would seem reasonable to expect that significant clinical advantage could be derived from several of the unique physiologic properties of fructose.

1. The almost exclusive utilization by hepatic tissue, the presence of a separate, specific and quite resistant set of hepatic enzymes for its disposal, and the rate of hepatic fructose uptake suggest its use as a carbohydrate source in liver disease. Thus when fructose is administered intravenously a much larger portion of the administered carbohydrate may be expected to enter the liver cells than in the case of glucose. This may well explain the relatively greater protein-sparing effect of fructose which has been described[33] and the favorable effect of fructose on some hepatic detoxifying processes, such as the striking effect in acute ethanolic intoxication.[34]

2. The rapidity of hepatic fructose uptake indicates its use as a substitute for glucose whenever glucose must be administered rapidly and whenever the renal threshold for glucose is exceeded, leading to glucose wastage and to the concomitant undesirable osmotic diuresis. Although the renal threshold for fructose is lower than that for glucose the rapid fall in blood fructose levels minimizes the amount of fructose lost in the urine. In the presence of untreated diabetes mellitus the markedly decreased glucose tolerance contrasts even more with the intact tolerance for fructose. Thus the addition of 2 or 5 per cent fructose to hypotonic balanced electrolyte solutions used during the initial treatment of diabetic acidosis has been suggested.[35] A much smaller portion of the carbohydrate thus administered is wasted in the urine than would be true for glucose.

3. A further rationale for the use of fructose during the first hours of treatment of diabetic acidosis should be mentioned. While glucose uptake by diabetic liver is markedly impaired,[36] direct effects of insulin on hepatic tissue have not been well established. In the rat it has been clearly demonstrated[37] that several hours elapse before insulin administration affects the deranged intermediary metabolism of the diabetic liver. Fructose, which can be immediately utilized by diabetic liver, may thus represent an emergency carbohydrate supplement.

4. Present knowledge does not permit a decision as to whether or not fructose has anything to offer when used as an adjunct in the long-term therapy of diabetes mellitus. It is difficult, however, to dismiss the thought that, since perfect control of diabetes with insulin throughout the twenty-four hour period is rarely achieved, fructose, representing a ready source of insulin-independent carbohydrate for the liver, might supply the liver with necessary carbohydrate molecules during daily periods of relative insulin deficiency. Whether this could contribute, for instance, to control of the anomalies of lipid metabolism in the diabetic liver is a question deserving some consideration. Certainly it would appear from recent trials in man[38] as well as in rats[39,40] that a somewhat greater portion of administered fructose is retained than is the case for glucose. It had previously been suggested that with prolonged periods of administration more and more fructose would be lost as glucose. These recent observations, however, do not appear to support this suggestion.

5. Finally, it should be mentioned that the rapid and almost exclusive hepatic utilization of fructose may be used to assess the functional state of the liver at any given moment by presenting it with a fructose load and by measuring the relative rates of appearance of various groups of its metabolites. Similarly, it has been shown in liver slices that although normal and diabetic tissue utilize fructose at the same overall rate, incorporation of fructose into different metabolites varies greatly with the metabolic situation encountered.[12]

POTENTIAL CLINICAL DANGERS

Certain potential clinical dangers should not be forgotten. First, whenever fructose is administered in large amounts the assumption is made that the liver will transform a sufficient amount of fructose into glucose in order to supply the needs of tissues such as brain and muscle which cannot use fructose directly in sufficient amounts. This assumption is not always justified. For instance, in patients receiving insulin the peripheral rate of glucose removal from the blood stream may exceed the rate of glucose formation from fructose, and hypoglycemia may develop. This may go unrecognized since routine methods for blood glucose measure fructose as well. Further difficulties may result from the excessive rate

at which fructose is metabolized when given intravenously in large amounts. Sufficient amounts of lactate and pyruvate may accumulate to produce significant acidosis. Also, in the treatment of diabetic acidosis glycogen deposition from fructose may be rapid enough to accentuate greatly any tendency to hypokalemia. Finally, it should be stressed that the administration of fructose in diabetic acidosis should never be considered to alter in any way the insulin requirement or the need for speed in reaching adequate control with insulin.

SUMMARY AND CONCLUSION

The metabolism of fructose has recently been greatly clarified and has been found to differ considerably from that of glucose. The main features of this difference are the almost exclusive utilization of fructose by liver, the presence of separate specific enzymes for its disposal, the rate of its uptake by hepatic tissue and the lack of any significant dependence of this rate on the presence of insulin. The abundant literature concerning its clinical usefulness which has accumulated over the past fifty years yields no conclusive answer. A fresh appraisal of the potential clinical usefulness of fructose in the light of new knowledge concerning its physiologic properties is clearly indicated.

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Clinical Studies

Precipitation of Ventricular Arrhythmias Due to Digitalis by Carbohydrate Administration*

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THE clinical studies of Sampson et al.[1] demonstrating the abolition of digitalis-induced ventricular arrhythmias by administration of potassium salts have been recently reinvestigated by Lown and coworkers,[2] and the relationship of digitalis intoxication to potassium metabolism has been extensively reviewed by Lown and Levine.[3,4] The latter were able to confirm Sampson's findings and in addition to establish that potassium depletion predisposes to the development of cardiac manifestations of digitalis toxicity and enhances arrhythmias already present. The so-called "postmercurial redigitalization phenomenon," the arrhythmias occurring in digitalized patients during therapy with adrenal steroids, and the similar cardiac disturbances in digitalized subjects sustaining large losses of gastrointestinal fluids were all shown to have a potassium deficit as their common denominator. Although no reliable correlation was found between sensitivity to digitalis and the serum potassium concentration, a reciprocal relationship was thought to exist between total body potassium and sensitivity to the foxglove: the tendency to cardiac arrhythmias during digitalis therapy was increased by potassium depletion, and the arrhythmias in turn were abolished by correction of the potassium deficit.

The occurrence postprandially of electrocardiographic changes resembling those of potassium deficiency (T wave depression, appearance of prominent U waves) with or without a fall in serum potassium levels has been described by Rochlin and Edwards.[5] These workers implicated dietary carbohydrate as responsible for the changes found. They suggested that the electrocardiographic or chemical evidence for a lowered potassium results from the entry of potassium with carbohydrate into liver and muscle cells, presumably through a mechanism involving endogenous insulin.

The demonstration of carbohydrate-induced hypopotassemia or of changes resembling potassium depletion, together with the now well documented aggravation of digitalis toxicity by potassium deficiency, suggested that a relationship might exist between carbohydrate metabolism and clinical digitalis intoxication. The following study will report seven patients in whom ventricular arrhythmias were apparently precipitated by orally or intravenously administered carbohydrate while they were being treated with digitalis glycosides.

METHODS

Patients who were receiving digitalis therapy or presented problems of special interest in digitalis intoxication were selected from the ward service of the Jefferson-Hillman Hospital. Thirty-seven patients were investigated a total of ninety-nine times. With the exception of J. T., who was followed as an outpatient, all patients were studied in the basal, fasting state at about 6:30 A.M. Continuous control electrocardiograms were taken for a three- to five-minute period. One hundred grams of glucose in water were then given orally in five minutes, and serial three-minute strips of electrocardiograms (using a Sanborn Viso-Cardiette) were taken at five- to fifteen-minute intervals for a period of from thirty to 120 minutes. Intravenous admin-

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istration of 50 cc. of 50 per cent glucose in water or of 200 to 400 cc. of 10 per cent glucose in water, or the eating of an ordinary high carbohydrate (low-salt) breakfast, were occasionally substituted for the test dose of oral glucose. The observer was present throughout the test to insure that subjects remained quiet, the usual result being that patients fell asleep during the procedure. Whenever possible, patients were studied repeatedly on successive days following stepwise increases in their digitalis dosage. No systematic attempt was made to follow serum potassium levels.

RESULTS

Of the thirty-seven patients in the group studied, ventricular arrhythmias attributable to carbohydrate administration were demonstrated in seven. Ventricular arrhythmias were precipitated on ten occasions after ingestion of 100 gm. of glucose by mouth, on three occasions after a high carbohydrate meal, once after rapid injection of 50 cc. 50 per cent glucose in water intravenously and once after slower infusion of 10 per cent glucose in water. Identical changes were produced by intravenously administered glucose and by a high carbohydrate meal in one patient; another patient demonstrated the same changes after oral glucose as after a high carbohydrate meal.

At the time of the procedure, five of the patients manifested either nausea or ventricular arrhythmias not present before digitalis therapy and would therefore, by the classic criteria, have been considered to show overt digitalis intoxication. In two patients the manifestations of toxicity, as reflected by ventricular premature beats, were evident only after administration of carbohydrate; both of these men had exhibited these arrhythmias in clear relationship to their digitalis therapy on previous days, although they were not considered on clinical grounds to exhibit digitalis toxicity at the time of the test. Of the thirty patients in whom carbohydrate administration failed to elicit disturbances of rhythm, digitalis intoxication was suspected on clinical grounds in only three. Two of these had ventricular premature beats not present before digitalis therapy; the third showed electrocardiographic evidence of auricular tachycardia with block developing while his digitalis dosage was being rapidly increased. Three patients in whom the test procedure induced arrhythmias were receiving 6 gm. or more of potassium chloride daily by mouth. None of these had taken potassium for at least eight hours before carbohydrate administration.

CASE REPORTS

CASE I. (Hospital No. 15942.) W. R., a fifty-nine year old man, was admitted with a history of exertional dyspnea and edema of three years' duration. Hypertension was known to have been present at least one year. The patient had received 0.1 gm. digitalis folia daily for the past twelve months. In the week preceding admission, as an outpatient, he was given four mercurial injections, supplemented by 0.3 gm. potassium chloride three times daily. There were no symptoms of digitalis intoxication.

The electrocardiogram showed only first degree heart block and non-specific T wave changes. A control tracing revealed one premature beat in five minutes. Sixteen minutes after oral administration of 100 gm. of glucose the tracing showed coupled, occasionally repetitive and multiform premature beats (Figs. 1A and B) with a frequency of twelve per minute, which were still present fifty-five minutes later. Similar results were obtained after oral glucose on three additional occasions, on which it was further noted that, while no premature beats could be demonstrated immediately after ingestion of glucose, bigeminy would invariably appear between fifteen and twenty minutes after such feedings.

To test the effect of an ordinary meal the patient was studied before and after an ordinary low-salt breakfast consisting of scrambled eggs, two pieces of toast, and weak coffee with a double serving of sugar. A five-minute control cardiogram and a three-minute strip immediately after eating showed no premature beats. Fifteen minutes after the end of the meal bigeminal beats were first apparent, increasing to a frequency of 5 per minute thirty minutes postprandially (Fig. 2) and still present after one hour.

CASE II. (Hospital No. C35198.) J. H., a fifty-five year old man, was admitted with a history of angina pectoris for nine months, and edema, dyspnea and orthopnea for three weeks prior to entry. Physical examination revealed a blood pressure of 130/90 and pulse of 85. The patient was a chronically ill appearing male with Cheyne-Stokes respirations, rales at both lung bases, hepatomegaly, and the thickened skin of chronic edema over his lower legs. The



FIG. 1. Case I. (Lead III.) A, control tracing before oral glucose showed only one premature beat in five minutes. B, sixteen minutes after 100 gm. glucose by mouth; note numerous bigeminal, multiform, occasionally repetitive premature beats.

FIG. 2. Case I. (Lead II.) A, control tracing before breakfast; no premature beats in five minutes. B, thirty minutes after breakfast; note appearance of bigeminy.

heart was enlarged to the anterior axillary line with a localized, expansile apical impulse, accentuated P2, diastolic gallop, and a grade I decrescendo early diastolic murmur along the left sternal border. Admission laboratory data included a serum potassium of 5.3 mEq./L., serum sodium of 145 mEq./L. The patient received 1.7 gm. digitalis folia over a nine-day period, and was given occasional mercurial injections, and a daily dose of ammonium chloride, 4 gm. per day. There were no symptoms of digitalis toxicity.

The electrocardiogram was suggestive of left ventricular hypertrophy and digitalis effect. A control tracing revealed multiform ventricular premature beats with a frequency of only two per minute. (Fig. 3A.) Five minutes after ingestion of 100 gm. of glucose the T waves became inverted. At thirty minutes the frequency of premature beats had increased to eight per minute. Forty minutes after ingestion of glucose there was marked cardiac slowing and the electrocardiogram showed the classic changes described in potassium deficiency, with prolongation of the P-R interval, inverted T waves, and prominent positive after-potential ("U waves"). (Fig. 3B.) Concomitantly, the frequency of premature beats increased to twelve per minute, the coupled premature beats appearing to interrupt the U wave. (Fig. 3C.) Repetition of this test on two additional days yielded essentially similar results.

CASE III. (Hospital No. A93439.) W. B. S., a sixty-six year old man, was admitted with a history of dyspnea of seventeen months' duration, and of edema and nocturnal dyspnea of three months' duration. Although he was known to be subject to bouts of "paroxysmal tachycardia," he had not been digitalized because of alleged "digitalis sensitivity." Physical examination revealed a blood pressure of 140/90 mm. Hg and a pulse of 95–100 per minute. The patient was a somewhat wasted, emphysematous man with distended neck veins, rales at both lung bases and hepatomegaly. The heart was questionably enlarged and, except for an irregular rate, was not otherwise remarkable.

Admission electrocardiogram (before digitalis) was suggestive of left ventricular hypertrophy and revealed ventricular premature beats from the same focus (Fig. 4A), often appearing as a trigeminal rhythm. It was found difficult to digitalize the patient because of a tendency to develop a bizarre, presumably ventricular tachycardia. He was finally given a total of 3.5 mg. gitaligen® over a three-day period and potassium chloride 6 gm. per day.

Electrocardiograms before and five minutes

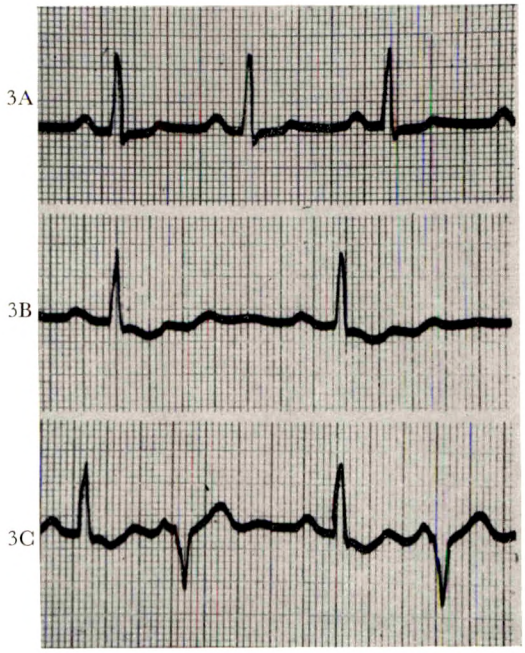


FIG. 3. Case II. (Lead II.) A, control tracing. B and C, strips from different parts of tracing taken forty minutes after 100 gm. glucose by mouth; note prolonged P-R interval, T wave changes and prominent "U waves" in B which are interrupted by coupled premature beats in C.

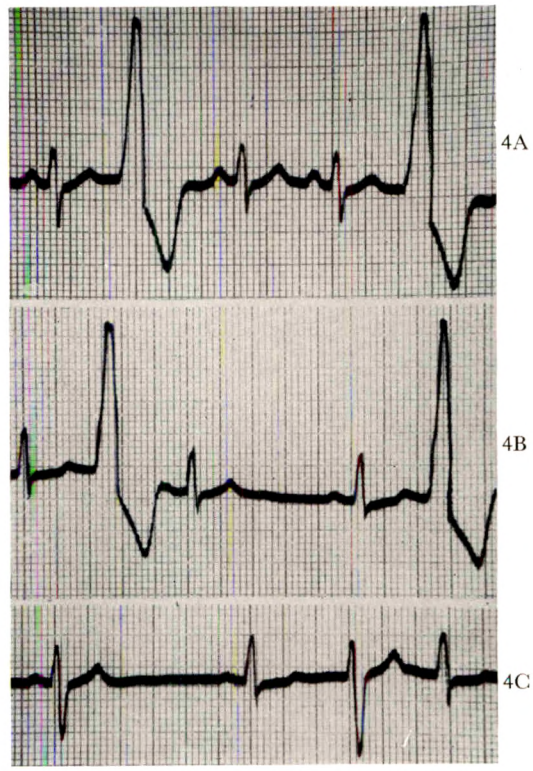


FIG. 4. Case III. (Lead II.) A, control, before digitalization. B, control before glucose, after digitalization; note persistence of bigeminal beats present before digitalis. C, twenty minutes after intravenous injection of 50 ml. of 50 per cent glucose in water; note replacement of previous bigeminy by ectopic beats of different configuration.

after intravenous injection of 50 cc. of 50 per cent glucose in water showed only the premature beats which had been noted prior to digitalization. (Fig. 4B.) Ten minutes after the injection the previously described premature beats were noted virtually to disappear, to be replaced by complexes from a new ectopic focus with a frequency of fifteen per minute (Fig. 4C) which persisted for forty-five minutes. In the subsequent twenty-four hours the patient received an additional 1.5 mg. of gitaligen. The next morning tracings taken before and immediately after breakfast (consisting of grapefruit juice, cereal with sugar, one egg and weak coffee with sugar) again showed only the single focus of premature beats that had been present before digitalization. Fifteen minutes postprandially the new ectopic focus previously elicited with intravenous glucose reappeared, reaching a maximum frequency of 16 per minute one-half hour after the meal, and again completely replacing the other focus.

CASE IV. (Hospital No. C35354.) H. H., a forty-eight year old man with known luetic aortic insufficiency, had been followed in the outpatient department over a six-month period on maintenance doses of digitalis, multiple mercurial injections, oral mercurials and carbonic acid anhydrase inhibitor. Two weeks prior to admission, on a visit to the emergency ward, he had inadvertently received a second complete digitalizing dose of digitalis leaf while continuing his maintenance dose of the drug and the mercurial diuretic therapy. On this regimen he had developed increasing edema and dyspnea and on admission had been vomiting for several days. Physical examination revealed a cachectic, orthopneic man with a blood pressure of 130/80 mm. Hg, distended neck veins, hepatomegaly and edema. The heart was diffusely enlarged to the right and left, the heart sounds tumultous with a rapid rate alternating with runs of slower bigeminal rhythm. Admission electrocardiogram revealed complete atrioventricular dissociation with multiform, coupled ventricular complexes and runs of multidirectional ventricular tachycardia at a rate of 150 beats per minute. An intravenous infusion of 5 per cent glucose with 40 mEq. potassium chloride



FIG. 5. Case IV. (Lead II.) A, control tracing showing complete A-V dissociation with normal ventricular complexes. B, twenty minutes after breakfast; note appearance of ventricular tachycardia.

FIG. 6. Case V. (Lead I.) A, control tracing showing only slow auricular fibrillation. B, fifteen minutes after start of infusion of 10 per cent glucose in water; note appearance of ventricular ectopic beats.

FIG. 7. Case VI. (Lead aVL.) A, control tracing shows no premature beats. B, forty-five minutes after 100 gm. glucose by mouth; note appearance of bigeminy.

promptly abolished the ventricular tachycardia and bigeminal beats, and reverted the configuration of the ventricular complexes to what it had been prior to the present episode. Termination of the infusion resulted in rapid recurrence of the chaotic ventricular rhythm. Accordingly, the patient was given procaine amide, 0.5 gm. every four hours by mouth, and potassium chloride, 2.0 gm. every eight hours. Thirty-six hours later a three-minute control tracing taken before breakfast revealed no abnormal ventricular complexes, although complete A-V dissociation persisted. (Fig. 5A.) The patient was then given breakfast consisting of toast, cereal with a double serving of sugar, eggs and weak coffee with sugar, taken over a half-hour period. A tracing taken five minutes after breakfast showed one premature beat in three minutes. Twenty minutes postprandially there appeared a one and one-half minute run of ventricular tachycardia at a rate of 110 per minute. (Fig. 5B.) Potassium and procaine amide therapy was continued for the next twenty-four hours. Feeding of 100 gm. of glucose the following morning failed to elicit abnormal ventricular complexes.

CASE V. (Hospital No. C42179.) C. P., a twenty-three year old man with rheumatic heart disease and congestive heart failure of long-standing, was admitted because of progressive dyspnea. Physical examination revealed cardiomegaly, auricular fibrillation, and murmurs of mitral stenosis, aortic stenosis and aortic insufficiency. An electrocardiogram showed auricular fibrillation, left ventricular hypertrophy and occasional ventricular premature

beats. These ventricular premature beats disappeared during intensified digitalis therapy. Two tests, on successive days, with 100 gm. glucose orally failed to elicit any arrhythmias after 1.6 gm. digitalis folia and 1.0 mg. digoxin, respectively. On the fifth day of digitalis therapy, having had a total of 2.2 gm. digitalis, the patient for the first time complained of nausea. Attempts at oral glucose feeding were aborted by retching. The patient was accordingly given an infusion of 10 per cent glucose in water at 15 cc. per minute. Control electrocardiogram (Fig. 6A) showed no abnormal ventricular complexes. The ventricular rate remained at seventy per minute throughout the infusion. Fifteen minutes after the start of the intravenous drip, tracings indicated multiple repetitive ventricular premature beats (Fig. 6B) (alternatively, a slightly irregular idioventricular pacemaker). Five hours later, when the patient's nausea had completely subsided, intravenous injection of 40 cc. of 50 per cent glucose in water failed to elicit abnormal ventricular complexes.

Case vi. (Hospital No. C21863.) L. M., a forty-six year old man with luetic aortic insufficiency, long-standing hypertension and congestive failure of four years' duration, was admitted because of increasing edema, anorexia and nausea. He had received 1.4 gm. of digitalis folia over a five-day period in addition to his previous maintenance dose. Physical examination revealed a blood pressure of 210/140 mm. Hg, marked wasting, pulmonary congestion, hepatomegaly, edema, marked cardiomegaly, and a grade iv aortic decrescendo diastolic blowing murmur. In spite of marked nausea the patient was able to retain 100 gm. of glucose by mouth. Control electrocardiogram showed auricular fibrillation, left ventricular hypertrophy and intraventricular block, but revealed no premature beats. (Fig. 7A.) Thirty minutes after glucose ingestion rare bigeminal beats appeared, and at forty-five minutes runs of classic coupling were in evidence. (Fig. 7B.)

Case vii. (Hospital No. 53054.) J. T., a seventy year old man with known hypertension for six years, was seen as an outpatient with complaints of nocturnal dyspnea and edema of six weeks' and one month's duration, respectively. Physical examination revealed an obese, emphysematous man with a blood pressure of 220/130 mm. Hg, distended neck veins, signs of fluid at the right lung base, dependent edema and moderate cardiomegaly. An electrocardiogram prior to digitalization showed first degree heart block and was suggestive of left ventricular hypertrophy. There were no premature beats. The patient received 1.2 gm. digitalis folia over a twenty-four-hour period. Following this he had a diuresis and diarrhea (seven stools in one day) developed. He was seen again thirty hours after the last dose of digitalis. Control electrocardiogram at this time revealed increase in his first degree heart block with occasional 2:1 second degree block. In addition, rare multiform ventricular premature beats had developed with a frequency of two per minute. Fifteen minutes after an oral dose of 100 gm. glucose the premature beats had become repetitive and more frequent, attaining a maximal frequency of fifteen per minute at one hour.

DISCUSSION

Clinical Implications. The precipitation of fatal ventricular arrhythmias is the cardinal hazard of digitalis therapy. Frequently the appearance of ventricular extrasystoles heralds the onset of an ominous sequence which, in the presence of the underlying structural heart disease, progresses to ventricular tachycardia and culminates in ventricular fibrillation. The demonstration that such arrhythmias can be brought out or aggravated by oral or intravenous carbohydrate administration is therefore of some importance. This is true in the patient nauseated from digitalis overdosage, in whom infusions of glucose in water are often the principal means of sustaining adequate nutrition while nausea and vomiting persist. It applies equally to the decompensated patient with a recent myocardial infarction (or some other cause of an "irritable myocardium") in whom the delicate balance may be upset in favor of ventricular fibrillation by a digitalis-induced ectopic rhythm.

If it is true that the arrhythmias described here represent aggravation of digitalis toxicity as a result of a fall in the plasma potassium secondary to fluctuations in the blood sugar level, clinical judgment might suggest the advisability of certain precautionary steps. Prophylactic administration of potassium salts with oral or intravenous carbohydrate, and avoidance of excessive blood sugar fluctuations by restricting highly refined carbohydrate in the diet would seem logical measures; it must be emphasized that no evidence for their efficacy is available from this study. In this regard, the

management of digitalis intoxication resembles that of periodic familial paralysis,[6] since in both conditions ventricular arrhythmias on the one hand, and muscular paralysis on the other, can be precipitated by a carbohydrate-induced serum potassium fall and can be prevented by potassium therapy. In view of the fact that both epinephrine and insulin have been shown to cause a marked fall in blood potassium levels,[7,8] one may speculate further on the importance of suppressing excessive emotional discharges and spontaneous hypoglycemia in critically digitalized patients.

Mechanism. Lown and associates were able to produce ventricular ectopic beats and ventricular tachycardia by the selective removal of potassium from digitalized dogs[9] and patients,[3] using the artificial kidney. In addition, they reported a patient in whom digitalis intoxication was evoked after lowering the serum potassium from 4.1 mEq./L. to 3.0 mEq./L. following administration of 50 gm. glucose with 25 units of insulin, the arrhythmia being abolished by a single oral dose of potassium acetate.[2] These data, in conjunction with their similar findings in potassium depletion associated with mercurial diuresis, gastrointestinal potassium loss and adrenal steroid therapy, have established that a fall in serum potassium or potassium depletion may bring out toxic cardiac manifestation due to digitalis in digitalized subjects.

In 1924 Harrop and Benedict[10] first demonstrated the fall in plasma potassium levels after oral glucose feeding of non-diabetics. Farber et al.,[8] who recently restudied this phenomenon, were able to show a consistent fall in *arterial* plasma potassium levels after oral or intravenous glucose, and emphasized the failure of the *venous* plasma potassium levels to reflect the changes detected in arterial blood. The latter finding may explain why Rochlin et al.,[5] who determined postprandial potassium levels in venous blood, were often unable to correlate the electrocardiographic changes suggestive of hypopotassemia with a simultaneous fall in potassium. That these electrocardiographic changes are clearly related to hypopotassemia was demonstrated by Parrish, Sugar and Fazekas,[11] who were able to reverse the T wave inversion and Q-T prolongation during insulin-induced hypoglycemia and hypopotassemia by administering potassium, without, at the same time, correcting the hypoglycemia. Moreover, the terms "postprandial T wave inversion, prolonged Q-T interval, and appearance of U waves" are probably inaccurate, since all of these electrocardiographic phenomena are said to represent interference with the T or U wave by a positive after-potential which is distinct from the T and U waves and which may be abolished by potassium administration.[12,13] There is good evidence[14] relating the plasma potassium fall to the entry of potassium into hepatic parenchymal cells during deposition of liver glycogen. Experiments *in vitro* on potassium entry into muscle during glucose deposition have yielded discordant results,[15–17] and studies in man[8] were interpreted by Farber et al. as pointing to a loss of potassium by peripheral tissues in an effort to maintain the plasma level. Whatever the mechanism, the arterial plasma potassium does fall. There is some question as to whether the electrocardiographic changes resembling those of potassium depletion are a reflection of the lowered level in the arterial plasma or of a total body potassium deficit. In the latter case, one would have to postulate that, in entering the cells with carbohydrate, potassium temporarily enters a "compartment" which is not functionally a part of ionically active body potassium.

An alternative explanation for the fall in plasma potassium would be to link it to the extracellular alkalosis of the postprandial "alkaline tide." This explanation receives some support from the demonstration by Magida and Roberts[18] that the electrocardiogram in alkalosis is indistinguishable from that of hypopotassemia, and from the finding by Darrow and others[19] that alkalosis tends to be associated with potassium depletion. Operation of this mechanism in precipitating digitalis intoxication by its potassium-lowering effect cannot be ruled out in those patients in this series manifesting toxicity after an ordinary meal, but seems unlikely after oral glucose alone, and is not applicable to those instances in which intravenous glucose was used. Similarly, an attempt to explain carbohydrate-induced ventricular arrhythmias in digitalized patients on the basis of the extra demands made on the circulatory reserve by the digestive processes fails to account for the response to intravenous glucose and would not explain why this reaction was found only in patients at or near the point of digitalis intoxication. All thirty-seven patients in the group studied had heart disease of comparable severity; in fact, while the seven patients with positive responses are still living, there have

been five deaths in the thirty patients in whom carbohydrate failed to induce ventricular arrhythmias.

SUMMARY

Administration of carbohydrate orally or intravenously in seven patients at or near the point of digitalis intoxication precipitated ventricular premature beats in six patients and ventricular tachycardia in one patient.

This effect is attributed to reduction in the arterial plasma potassium level after carbohydrate administration.

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The Efficacy of Carotid Sinus Pressure in the Differential Diagnosis of Triple Rhythms*

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THE accurate diagnosis of heart disease depends to a considerable extent upon the art of auscultation first described over 2,000 years ago by Hippocrates;[1] brought into sharp focus with the introduction of the stethoscope by Laennec in 1819;[2] first graphically recorded by Huerthle[3] in 1893; its basic laws defined by Rappaport and Sprague[4,5] in 1941–1942; and owing many of its present achievements to the important contributions of Levine,[6,7] Wolferth and Margolies,[8] Evans,[9,10] Lian,[11] Luisada[12] and others.[13–18]

Normally, the contraction and relaxation of the heart produces two sounds; occasionally, three. These are predominantly valvular in origin, the energy of the first sound being derived primarily from vibrations set up by the closure of the A-V valves and that of the second sound from vibrations subsequent to the closure of the semilunar valves. Muscular and vascular movements also contribute to these sounds so that the graphic record of each sound can actually be divided into four component parts on the basis of their derivation. In conditions which alter the valves, vibrations result which have been termed murmurs and whose significance and clinical interpretation forms an integral part of the art of auscultation.

In conditions which alter muscular function, notably left or right ventricular failure, an additional sound may appear during diastole which is commonly termed a gallop sound and the rhythm referred to as a gallop rhythm, from its resemblance to the canter of a galloping horse.[13,19,20] The phenomenon has achieved clinical significance in that it is almost invariably associated with a failing ventricle and tachycardia. Supposedly, it rarely if ever is audible at rates below 100. A gallop sound consists of usually one to seven coarse vibrations of very low frequency as compared to a murmur which consists of a series of finer, more regular and more numerous vibrations. Evans[9] has given an excellent graphic differentiation of sounds versus murmurs. He states that in the case of a sound the vibration is coarse and the distance between each vibration forms a square in relation to a millimeter scale. In the case of a murmur the vibrations are finer and form an oblong. Additional sounds may occur during systole but these are usually of no real clinical significance and therefore should not be called gallop sounds. Extra sounds during both diastole and systole may be confused with splitting or reduplication of the first or second heart sound, particularly with rapid rates.

The term triple rhythm, or three-sound rhythm, has been introduced in order to encompass conveniently all of these various extra heart sounds. Our classification, based upon the work of a number of authors, is as follows:

I. Diastolic sounds
  A. Pathologic
    1. Protodiastolic gallop rhythm
    2. Presystolic gallop rhythm
    3. Summation gallop rhythm
    4. Opening snap of mitral stenosis
  B. Physiologic
    1. Protodiastolic sound (third heart sound)
    2. Presystolic sound (auricular sound)
    3. Fusion sound (Wolferth and Margolies[22])
II. Systolic Sounds
  A. Pathologic
    1. Pulmonary early systolic sound (dilatation of the pulmonary artery, Leatham and Vogelpoel[23])

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B. Non-pathologic
   1. Systolic click, tap, knock, scratch, etc.
   2. Systolic sounds
      (*a*) Aortic systolic sound
      (*b*) Apical systolic sound
   3. Reduplication of normal heart sounds

Protodiastolic Gallop and Physiologic Third Heart Sound.[12,22,24–31] The protodiastolic gallop sound, or accentuated third heart sound, occurs early in diastole during the first phase of ventricular filling, or that of "rapid passive filling." Since the timing is identical whether the sound is pathologic or physiologic, the gallop and third heart sound will be described together.

The best description of the third heart sound is that written by William Thayer[24] in 1908. He was able to hear a third heart sound in 65 per cent, of young people with thin chest walls. He states: "This sound is softer and of lower pitch than the second sound; sometimes it is a dull, distant thud; sometimes merely a hum; it occurs shortly after the second sound, i.e., at the beginning of the long pause. It is, however, always appreciable at a time or later than that occupied by a true reduplication of the second sound, and it is audible only or with greatest intensity at or near the apex. In some cases it may be heard over the lower part of the right ventricle. At times, the sound is followed by the faintest suggestion of an echo resembling a slight early mitral diastolic murmur. . . . The quality of the sound is low pitched and clear with no suspicion of harshness or anything suggesting a murmur." He observed that the sound occurred at the beginning of the protodiastolic wave of the apex cardiogram, and simultaneous with the h wave of the jugular pulse tracing or immediately after the fall of the v wave. The sound was best heard with the patient on his back or left side during the short interval between expiration and inspiration. His cases all had relatively slow pulse rates (50–88). It was his impression that the sound was clear as compared to the dull sound of a gallop and distinguishable from the opening snap of the mitral valve with its short snapping quality. Wolferth and Margolies[22] observed no essential differences in the quality, location, time relations or influence of posture or various maneuvers to alter the sounds in distinguishing gallop from physiologic third heart sounds. They found that aside from the decreasing frequency with advancing age the only differential criterion was the status of the cardiac function. Bramwell[27] emphasizes that the physiologic third heart sound occurs without evidence of abnormality of cardiac function. He presents several differential points, namely: (1) gallop sounds are easily palpable as well as audible (except in emphysematous patients); (2) the accessory sound in gallop rhythm is only a dull thud, an abnormality of rhythm often more obvious on palpation; (3) the normal impulse produces a single thrust but the gallop impulse is a double wave with two components similar to each other, and (4) the sounds in gallop rhythm are almost evenly spaced whereas the physiologic third heart sound is obviously more closely related to the preceding second sound. The sound usually consists of one slow vibration which follows the second sound by 0.10 to 0.21 seconds (usually 0.13 to 0.18). If the second sound is split, the time relationship is maintained with one of the components. With reference to other phenomena, Luisada[12] states that it coincides with the termination of rapid relaxation in the electrokymogram of the left ventricular wall and slightly precedes the peak of the L wave of the ballistocardiogram. It has an average duration of 0.059 seconds but may vary from 0.050 to 0.061 seconds.

The physiologic third heart sound has been said to arise from vibrations of the A-V valves. It can be heard on direct auscultation of the heart in the open chest. The protodiastolic gallop supposedly results from the very rapid inflow of blood during early diastole into a dilated and flabby ventricle whose distensibility has been altered. The sound apparently arises from vibrations set up by the impact of the ventricle against the chest wall. Elevated pulmonary venous and left auricular pressure in the presence of an accelerated heart rate are factors which intensify this phase of rapid filling and predispose to the protodiastolic gallop. The sound may be heard in the presence of auricular fibrillation since its genesis does not depend upon auricular contraction.

Presystolic Gallop.[27–32] The presystolic gallop sound presumably represents an accentuated auricular sound. Its position actually depends upon the time of auricular contraction, but the sound usually occurs 0.08 to 0.14 seconds after the beginning of the P wave of the electrocardiogram. The average time of onset is 0.05

seconds after the peak of the P wave. Thus with A-V conduction defects the extra sound may precede the first sound by a considerably longer period, depending upon the P-R interval. In the case of a shortened P-R interval the split between the gallop and the first sound may be no wider than that between a reduplicated first sound. In view of these observations, Wolferth and Margolies point out that a wide split between the extra sound and the first sound favors a gallop whereas a narrow split has no differential value. Lian[21] refers to the exceptional situation in which the silent interval between the gallop sound and the first sound is lacking. He terms this a delayed presystolic gallop (galop présystolique retardé). The differentiation between this sound and a split first sound depends on whether the double sound precedes the summit of the R wave of the electrocardiogram or whether both vibrations follow the summit of the R wave, as is the case in splitting of the first heart sound. With reference to other time factors, the presystolic gallop sound occurs at the same time as a large distinct a wave of the apex cardiogram and the a wave of the jugular pulse. The auricular wave of the apex cardiogram is of large size in the case of a presystolic gallop.[31] It also coincides with point G of the ballistocardiogram and a small positive atrial wave of the electrokymogram of the left ventricle and with the descending limb of the negative atrial wave of the electrokymogram from the right or left atrial walls.[12] It is obviously not heard in the presence of auricular fibrillation. The sound is recorded best at the apex or midprecordium, especially in the left decubitus position and also in the epigastrium. It occurs during the peak of the third phase of ventricular diastole or the period of "rapid active filling" due to auricular contraction. Bond[31] presents an excellent discussion of the dynamic factors concerned with the production of this extra sound. He states, "As long as the damping action of the ventricle is preserved, auricular contractions remain inaudible. If, however, the ventricle has lost its silencing power, the reason for this change can only be an alteration in the state of its wall. (Amount of damping is proportional to the yielding capacity of normal ventricular muscle in diastole.) We must assume that the ventricular wall is no longer in a state of complete relaxation in diastole, but now offers a marked resistance to the entrance of blood during auricular systole.

"The pressure wave produced by the contracting auricle meets with a ventricular wall which no longer yields passively to every impulse; the pressure is raised suddenly in the large common cavity comprising both auricle and ventricle, and the whole of its surrounding wall is thrown into tranverse vibrations. These, transmitted to the wall of the chest, are heard as the familiar third sound of gallop rhythm. As has often been stated, it is best heard near the apex, which is that portion of the vibrating muscle mass closest to the wall of the chest. In this conception, the extra sound is a combined auricular and ventricular effect, and the participation of the ventricle explains why a sound, auricular in origin, should be so well perceived over the ventricle." Thus increased diastolic tension of the ventricle and increased intra-auricular pressure are regarded as the main factors in the causation of the extra sound.

In patients with normal rhythm or normal hearts, no distinct auricular sound is audible. In heart block, however, sounds synchronous with isolated auricular contractions may be heard. The first sound may be reinforced or accentuated in cycles where the extrasystole happens to synchronize with auricular contraction.

In 1888, Rouchès[33] introduced the term, "claquement d'ouverture de la mitrale" or opening snap of the mitral valve. He suggested that the sound was produced by the sudden stretching of a stenosed mitral valve as the blood rushed from left auricle to left ventricle. This extra sound has acquired added significance[35] with the advent of mitral valve surgery since it indicates the presence of a flexible or operable valve. It is invariably accompanied by a snapping mitral first sound. It has been observed that this sound is generally not present in the event of gross calcification of the valve, slight stenosis or marked insufficiency. Mounsey[34] made a clinical and phonocardiographic study of thirty-three patients with mitral stenosis as their predominant valvular lesion. The opening snap was heard in twenty-eight cases, in four it was seen only on phonocardiography, and in one the sound could not be elicited. The sound is composed chiefly of high frequency vibrations of brief duration (0.005 to 0.01 seconds). It was best heard over the supramammary area although in most cases it was heard equally well along the left sternal border and over the mitral area, least often over the pulmonic area. The average interval between the beginning of the

second sound and the opening snap was 0.07 seconds with a range of 0.03 to 0.14 seconds. He found that this distance varied directly as the length of the diastolic interval. In twenty-seven of the thirty-two cases the mitral snap was followed closely by a mid-diastolic murmur. In thirty-one of the thirty-three patients both splitting of the second sound and an opening snap were present. In every instance the split sound was heard loudest in the pulmonic area. The third sound is of much lower frequency than the opening snap. A helpful differential point in patients with physiologic splitting was the widening or disappearance of the split with deep inspiration. Such variation does not occur with splitting in bundle branch block. The opening snap coincides with the beginning of the descent of the v wave of the jugular pulse.

Summation Gallop.[22] In the event that the time zones of both protodiastolic and presystolic gallop sounds merge, an augmented or summation sound may be produced. This situation occurs in cases of tachycardia, A-V block, sinus arrhythmia or auricular extrasystoles. The sound may occur in failure but also in normal persons with tachycardia or in patients with rheumatic fever who exhibit tachycardia and prolonged P-R intervals. Wolferth and Margolies[22] in their series observed that the critical rate (rate at which the two extra sounds merge) was usually between 100 to 130 beats per minute. If the P-R interval was prolonged, however, the critical rate tended to be lower (90–100).

Systolic Sounds.[23,36–42] Leatham and Vogelpoel[23] recently described perhaps the only systolic sound considered pathognomonic of heart disease; namely, a pulmonary early systolic sound in patients with dilatation of the pulmonary artery. This sound was originally described by Lian and co-workers in 1937 and 1942 as an early systolic sound (claquement protosystolique) heard over the pulmonary area in patients with pulmonary stenosis and with dilatation of the pulmonary artery, and also at the aortic area in patients with aortic valve disease. Leatham and Vogelpoel performed detailed studies of this sound combined with catheterization of the pulmonary artery. The sound was found to be high-pitched and often sharp like a click. It was loudest over the pulmonic area and during expiration, at times almost disappearing during inspiration. It is not affected by position and has a constant place in the cardiac cycle. The sound gives the impression of wide splitting of the first heart sound but has more of a click-like quality. The time interval between the onset of major high frequency components of the first sound and the onset of the pulmonic extra sound averages 0.07 seconds with a range of 0.05 to 0.14 seconds. Of fifty patients demonstrating this sign on auscultation forty-four had pulmonary hypertension which was confirmed by cardiac catheterization in forty-two. Sixteen of the patients had mitral stenosis, seven had a patent ductus arteriosus, ten had a ventricular septal defect, seven had an atrial septal defect, three had primary pulmonary hypertension, and one had transposition of the great vessels. In the seventeen cases with patent ductus and ventricular septal defect the pulmonary pressure was apparently high enough to reverse the usual left-to-right shunt and thus abolish or diminish the continuous or long systolic murmurs, respectively. The authors state that the presence of this sound in patients with mitral stenosis indicates a severe degree of pulmonary hypertension, but that its absence does not mean lower pressures.

The so-called systolic gallop was first described by Cuffer and Barbillon[19] in 1887 as "mesosystolic gallop." They observed the sound in four patients with typhoid fever and in two with tuberculosis. Potain[13] stated that the sound could be heard best over the aortic area and regarded it as an arterial phenomenon subsequent to diminished elasticity and tonicity of the aorta. Wolferth and Margolies[36] were able to make the diagnosis of systolic gallop eight times in ten years despite the fact that they were particularly interested in the study of extra heart sounds. Thompson and Levine[37] observed thirty-five cases over an eleven-year period and during the same interval observed 186 patients with diastolic gallop rhythm. Their incidence of systolic gallop was therefore 16 per cent. They found that the extra sound resembled the first heart sound although the intensity was variable. The sound was heard only at the apex or with maximum intensity there in most patients. The patient's position also considerably influenced the intensity of the vibrations. The heart rate in most cases ranged from 70 to 80 beats per minute. These authors emphasize the benign nature of the sign which for the most part was found in "nervous people," two-thirds of whom had no evidence of heart disease. During the period in which their patients were observed 46 per cent of those with diastolic gal-

lop rhythm died or were presumed to have died whereas none of the patients with systolic gallop expired. After reviewing the many theories of genesis in the older literature, they summarized that, "Opinion as to mechanism is then roughly divided into two basic ideas, that of a lengthened period of isometric contraction of the ventricle with resultant dissociation of the elements of the normal first sound and that of production by a diseased or weakened aortic wall." White[43] mentions that the cause is unknown but may possibly be due to deformity of valves, chordae tendinae, pericardium or pleura. Wolferth and Margolies[36] suggest that the sound may arise from the pressure of the contracting heart against some emphysematous area in the overlying lung. They recognize two types of systolic gallop rhythm exclusive of the sounds which may fall between the first and second heart sound; namely, aortic and apical systolic gallop rhythm. They further state that the mid-systolic click bears no resemblance to the systolic gallop except that it falls between the first and second sound. It may have three or more components and is usually heard best near the apex.

The mid-systolic click is so described because of its obvious clicking quality. The quality of the sound has been variously described as rough, superficial, dry, vibrant, grating, tapping, etc. It is highly susceptible to positional and respiratory variations. The extracardiac origin of the sound has been very clearly demonstrated by Becker and Halpern[41] who found that the sound was not constantly related to any part of the cardiac cycle as determined by simultaneous jugular, carotid pulse and electrocardiographic tracings. In their case the sound varied from mid-systole to protodiastole in time. It is a completely benign but often striking sound and must occasionally be differentiated from a diastolic gallop or mitral opening snap.

Our observations with respect to gallop rhythm parallel those of Bramwell.[27] They are predicated upon the thesis that if the accepted physiologic explanations for true gallop rhythm are valid, then, with few exceptions, the demonstration of such a sound should be synonomous with the demonstration of heart failure. Debate as to the advisability of applying the term gallop rhythm to those cases with diastolic sounds which are inaudible and can be elicited only by graphic registration would seem to misinterpret the clinical connotation of this important sign. The application of such terms as triple rhythm to the over-all group of extra sounds is helpful.

One of the authors (W. B. P.) has found a simple bedside technic to be extremely helpful in the differentiation of gallop rhythm from other types of triple rhythm in approximately three-fourths of the patients who exhibit this sign. It has been observed that consequent to the slowing of ventricular rate effected by carotid sinus pressure, a true gallop sound will invariably become inaudible or disappear. The observation that variations in rate have a pronounced effect on gallop rhythm is not new; however, to our knowledge the utilization of a clinical test based on this fact for the simple and accurate differentiation of true gallop rhythm from other types of triple rhythm has not been generally practiced.

We have found the right carotid sinus to be almost invariably more effective than the left in producing slowing of the ventricular rate.

Ferris et al.[44] found no particular predominance of either sinus in reproducing the symptoms of the "cerebral" type of carotid sinus syncope, nor did Weiss and Baker.[45] Our observations would suggest that the right carotid sinus pressure is more effective in slowing the ventricular rate in patients with gallop rhythm.

MATERIAL AND METHODS

Simultaneous electrocardiograms and phonocardiograms were obtained from a series of thirteen consecutive patients who demonstrated a distinct gallop rhythm. In order to test the validity of the procedure, similar studies were carried out in four patients with complete bundle branch block, one patient with physiologic splitting, and five patients with extra sounds during systole. In three patients, right, left and bilateral carotid sinus pressure failed to produce a significant slowing of the ventricular rate. One of the three (L. M.) did, however, develop a slow rate with disappearance of his gallop rhythm following bilateral compression of his eyeballs (oculocardiac reflex). A tabulation of these cases is presented in Tables I and II.

CASE REPORTS

Figure 1 is the phonocardiogram of J. L. (Table I), a sixty-eight year old Negro man, who entered the hospital because of moderate hypertension complicated by early left ventricular failure. Auscultation revealed a diastolic

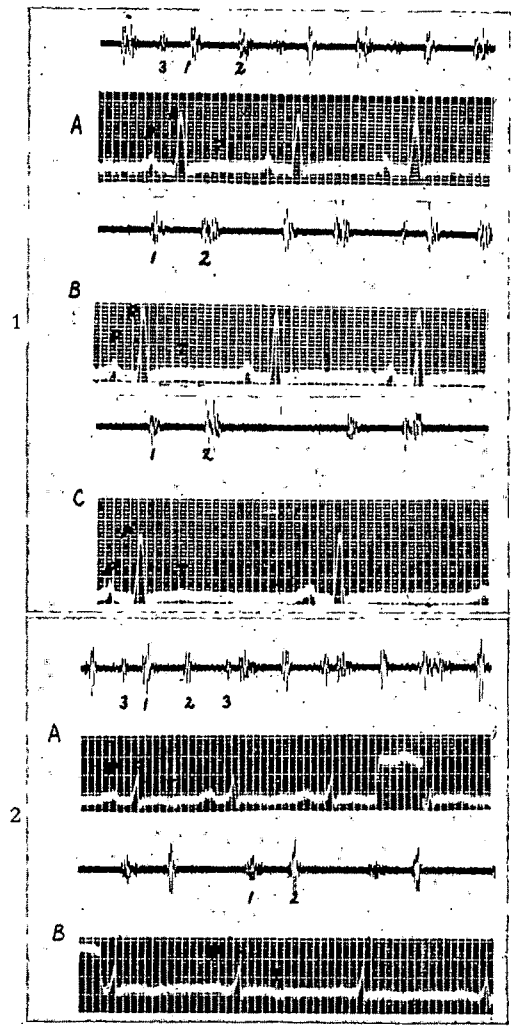


FIG. 1. Apical phonocardiogram from J. L. reveals a mid-diastolic gallop in A, which consists of one coarse vibration merging with three finer vibrations. Following right carotid sinus pressure (between A and B) the heart rate slowed from 105 to 60 beats per minute in C. In B, between the third first heart sound and the preceding second heart sound, the single coarse vibration has separated from the three finer vibrations, indicating the presence of a summation gallop in A. This separation occurs at a rate of 83. The finer vibrations follow the peak of the third P wave in B by an interval of 0.04 seconds and the single coarse vibration follows the preceding second sound by an interval of 0.11 seconds. The gallop sound became inaudible when the rate slowed below approximately 92 beats per minute.

FIG. 2. Phonocardiogram from E. F. reveals a presystolic gallop sound in A. The ventricular rate is 118. Following right carotid sinus pressure, the heart rate slowed to 75. The gallop sound is no longer visible in B with the rate slowed to 90 and became inaudible with a rate of approximately 95.

gallop rhythm. Electrocardiogram was interpreted as sinus tachycardia and left ventricular strain. Phonocardiogram from the apical region revealed one coarse diphasic vibration merging with three finer vibrations (Fig. 1, A). The ventricular rate at this point was 105. Following pressure on the right carotid sinus, the ventricular rate slowed to 60. With a rate of 83 the single coarse vibration separated from the three finer vibrations so that in B, Figure 1, the finer vibrations can be seen to follow the peak of the third P wave by an interval of 0.04 seconds and the single coarse vibration to follow the preceding second sound by an interval of 0.11 seconds. These sounds were inaudible when the rate slowed below approximately 92 beats per minute.

Figure 2 is the phonocardiogram of E. F. (Table 1), a forty-year old Negro man, who entered the hospital because of anasarca and congestive heart failure. He was a known alcoholic. Auscultation of the heart revealed a diastolic gallop rhythm. Despite the presence of marked cardiac decompensation, his arm-to-tongue circulation time using decholin was but ten seconds. He responded dramatically to thiamine. His electrocardiogram was interpreted as sinus tachycardia, low voltage and diffuse T wave abnormality consistent with diffuse myocardial disease. In A, Figure 2, are seen three medium coarse vibrations, presystolic in time. The ventricular rate is 118. Following right carotid sinus pressure, B, the gallop sound became inaudible and was no longer visible in the phonocardiogram. The sound became inaudible at a rate of approximately 95.

Figure 3 is the phonocardiogram of E. B. (see Table 1), a thirty-eight year old Negro man, who entered the hospital because of congestive heart failure. Auscultation revealed a diastolic gallop rhythm, which disappeared with ventricular slowing incident to pressure on the right carotid sinus. Electrocardiograms were interpreted as showing slightly prolonged intraventricular conduction and left ventricular strain. Because of failure to demonstrate an etiology for his generalized cardiomegaly he was diagnosed as having idiopathic cardiac hypertrophy. Phonocardiogram revealed a presystolic gallop sound consisting of two coarse vibrations separated by one finer vibration, A. The initial heart rate was 120. Following carotid sinus pressure, B, these vibrations disappeared from

TABLE I

GALLOP RHYTHM

Case No.	Patient	Age	Cardiac Diagnosis	Initial Heart Rate	Heart Rate When Gallop Inaudible	Type Gallop	Heart Failure Present
1	E. B.	38	Idiopathic hypertrophy	120	90	Presystolic	Yes
2	A. C.	27	Cor pulmonale	130	85	Summation	Yes
3	E. F.	40	Beri-beri	118	94	Presystolic	Yes
4	W. L.	66	Coronary	120	..	Mid-diastolic	Yes
5	J. L.	68	Hypertensive	105	92	Summation	Yes
6	L. M.	35	Hypertensive	120	98	Presystolic	Yes
7	C. M.	49	Coronary	125	..	Mid-diastolic	Yes
8	C. W.	31	Idiopathic hypertrophy	95	90	Presystolic	Yes
9	T. F.	27	Hypertensive	110	98	Presystolic	Yes
10	L. H.	44	Cor pulmonale	105	90	Presystolic	Yes
11	C. C.	26	Hypertensive	115	95	Presystolic	Yes
12	F. O.	41	Hypertensive (LBBB)	98	85	Presystolic	Yes
13	J. T.	59	Hypertensive (RBBB)	110	85	Presystolic	Yes

TABLE II

Case No.	Patient	Age	Diagnosis	Initial Rate	Maximum Slowing	Type	QRS Interval (sec.)	Variation in Position of Sound	Heart Failure Present
			A. Bundle Branch Block						
1	E. B.	61	Lung carcinoma	70	60	RBBB	0.13		No
2	F. O	41	Hypertension	98	80	LBBB	0.13		Yes
3	W. P.	60	Scleroderma	65	50	LBBB	0.14		Yes
4	J. P.	38	Rheumatic fever	87	67	RBBB	0.15		No
5	J. T.	59	Hypertension	110	85	RBBB	0.16		Yes
6	T. P.	30	Idiopathic hypertrophy	...	..	Physiologic splitting	0.08		Yes
			B. Systolic Sounds						
1	J. H.	44	Pleura carcinoma	102	70	Click		Yes	No
2	H. G.	56	Psychoneurosis	90	65	Click		Yes	No
3	E. B.	58	Emphysema	100	80	Click		Yes	No
4	S. T.	58	Cirrhosis	88	60	Apical "gallop"		No	No
5	A. S.	65	Cerebrovascular hemorrhage	65	40	Apical "gallop"		No	No

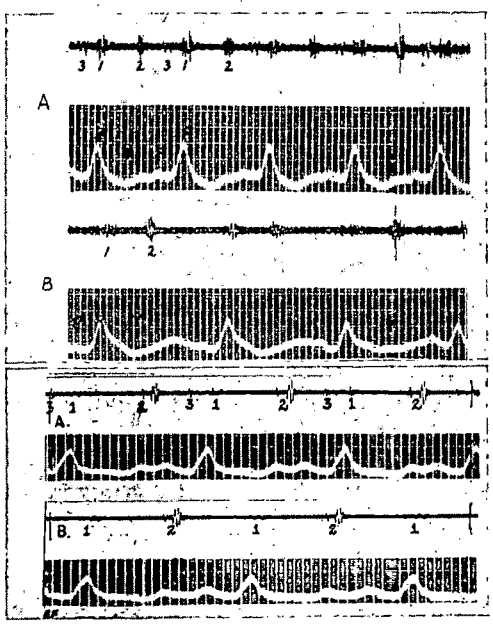


FIG. 3. Phonocardiogram from E. B. reveals a presystolic gallop sound in A with an initial heart rate of 120. Following right carotid sinus pressure in B, these vibrations disappeared from the sound tracing. The gallop sound became inaudible at a rate of approximately 90.

FIG. 4. An apex phonocardiogram from F. O. reveals a reduplication of the second sound with the two components separated by an interval of 0.05 seconds. The simultaneous electrocardiogram reveals a complete bundle branch block (left). A presystolic sound consisting of three coarse vibrations follows the peak of the P wave by 0.06 seconds. The initial heart rate, A, is 98. Following right carotid sinus pressure, B, the ventricular rate has slowed to 84 and the presystolic gallop sound is neither audible, nor visible on the sound tracing.

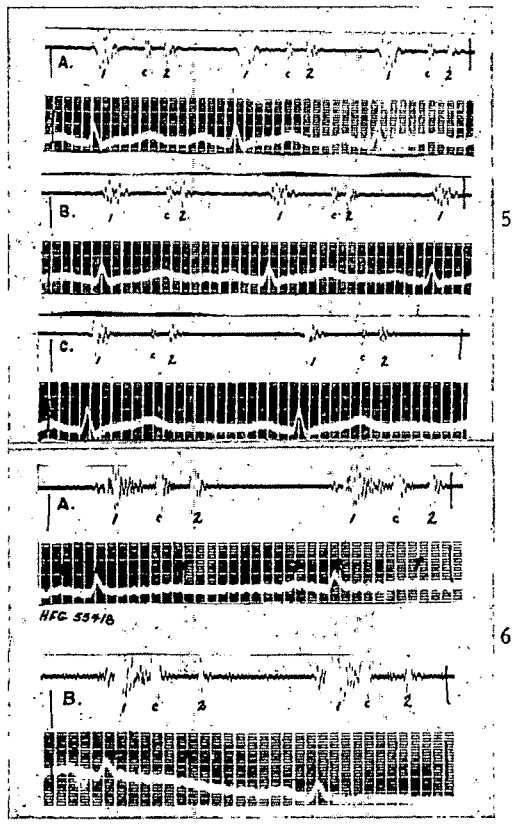


FIG. 5. Phonocardiogram from J. H. reveals a slight systolic murmur and a mid-systolic click (*c*). The ventricular rate in A is 101. Following carotid sinus pressure the ventricular rate has slowed to 90 in B and to 73 in C. It can be observed that the click approaches closer to the second sound in B and diminishes in intensity in C.

FIG. 6. Phonocardiogram recorded from H. G. reveals a split first sound. The second component of the split first sound is obscured by a moderate systolic murmur, which extends into mid-systole where a loud systolic click is evident. In A the rate is 70 and the click occupies a position in mid-systole. In B the rate is 85 and the click has shifted toward the first sound blending with the systolic murmur.

the sound record. The extra sound became inaudible at a rate of approximately 90.

One patient (L. M., Table I) failed to slow his ventricular response following bilateral carotid sinus pressure; however, his rate slowed quickly from 120 to 85 following bilateral eyeball (oculocardiac reflex) pressure. Another patient, C. W., responded with only a very slight decrease in ventricular response (8 beats per minute). His extra sound became inaudible with a decline in rate from 95 to 90. Although inaudible on auscultation, a faint presystolic sound was still apparent in the sound tracing after this slowing. Two patients with coronary heart disease and intractable failure demonstrated loud mid-diastolic gallop sounds but failed to alter their rapid ventricular rates either with bilateral carotid sinus pressure or bilateral eyeball pressure.

Two patients with complete bundle branch block had, in addition to a presystolic gallop, wide splitting of the second heart sound. The gallop sound disappeared following carotid sinus pressure without apparent change in the reduplication of the second sound. Figure 4 is the sound tracing from one of these patients, F. O. (Tables I and II), who represents one of the cases already described. The sound tracing revealed a presystolic gallop sound consisting of

three coarse vibrations, followed by a very faint prolonged first sound, and a reduplicated second sound. The rate slowed to 80 following carotid sinus pressure. The extra sound became inaudible with a rate of 84. With slowing, the reduplication was more evident with only a very slight change in the interval between the split sounds. Figures 5 and 6, J. H. and H. G., represent two examples of the so-called systolic click. H. G. has in addition a systolic murmur. This sound is easily differentiated by its characteristic "clicking" quality and the marked variation in the position which it occupies in the cardiac cycle with changes in rate, position and respiration.

COMMENTS

The use of carotid sinus pressure and the subsequent slowing of ventricular rate has been effective in rendering the gallop sound inaudible in eleven of thirteen patients, or 85 per cent of the cases reported upon. It has also been a valuable adjunct in the differential diagnosis of other types of triple rhythms. In keeping with the observations of King,[38,39] the authors have observed that the phenomenon which bears his name, viz., the reduplicated sounds which result from asynchronous contraction of the ventricles (and therefore asynchronous closure of the semilunar valves) in bundle branch block are invariably feeble or distant. Exceptions to this observation have been demonstrated only in young or very thin-chested individuals and in those with a gallop sound also present. We have further observed that bundle branch block is usually associated with a slow ventricular rate. Often the asynchronism of the apex thrust may be more obvious than the sound. In contrast, the gallop sound is usually quite loud and forms a characteristic rhythm or canter in which the three sounds are rather evenly separated. Like bundle branch block, it has been stated that the gallop rhythm is often more apparent on palpation than on auscultation. The heart rate with the true gallop rhythm is invariably rapid and the sound is low pitched and dull compared with the sounds produced by reduplication which obviously resemble the valvular sounds. Systolic sounds may be heard with a slow or rapid rate and must therefore be differentiated from a diastolic gallop or reduplication. It is at times difficult to differentiate the so-called "systolic gallop" from a short mid-systolic murmur. This sound is rarely heard in patients with heart disease, and when the combination does occur represents only an incidental finding.

Although the gallop sound was present at rates above 100 in all but two patients, C. W. and F. O., the "critical rate," or rate at which the gallop became inaudible by auscultation, varied between 85 and 98. The average critical rate for the eleven patients who responded to carotid sinus pressure was 91. The heart rate in the thirteen patients with gallop rhythm varied from 95 to 130 with an average rate of 113. It was observed that the extra sound vibrations were still visible on the phonocardiogram after the sound had become inaudible. In most cases the extra sound vibrations did not disappear from the phonocardiogram until the ventricular rate had slowed approximately 5 to 10 beats below the rate where the sound first became inaudible.

Clinical observations pertinent to the differential diagnosis of triple rhythms are tabulated in Table III. It can be seen that the true diastolic gallop sounds are invariably associated with a rapid ventricular rate and are usually of moderate intensity as compared to most other accessory sounds. The intensity is subject to slight respiratory variation. Although their timing is usually obvious on the phonocardiogram, on auscultation they appear to be more evenly spaced, certainly with respect to other three-sound rhythms. The apical impulse is frequently double and the rhythm is thus sometimes more apparent to palpation. The sounds are of very low frequency and usually consist of one to three vibrations, rarely as many as seven.

The opening snap of the mitral valve is usually loud and of higher frequency than a true gallop although not so high as a click. It is heard best in the supramammary area; however, it is generally audible with equal intensity along the left sternal border or over the mitral area, more rarely over the pulmonary area. An increase in the diastolic interval with slowing of the heart rate is accompanied by a longer interval between the beginning of the second sound and the opening snap. The snap usually follows a split second sound, which sometimes makes differentiation difficult. The split second sound, however, is invariably loudest over the pulmonary area. Inspiration tends to lengthen the interval between the split sound whereas expiration shortens it. In contrast, the sound of the opening snap may vary its intensity with respiration but not its timing. A physiologic

TABLE III

TYPE OF ACCESSORY SOUND.	PROTODIASTOLIC GALLOP	PRESYSTOLIC GALLOP	SUMMATION GALLOP	OPENING SNAP OF M. S.	PROTODIASTOLIC SOUND	PRESYSTOLIC SOUND	FUSION SOUND	PULMONARY EARLY SYSTOLIC CLICK	MID-SYSTOLIC CLICK	AORTIC SYSTOLIC CLICK	BBB WITH REDUPLICATION	PHYSIOLOGICAL SPLITTING
EFFECT OF CAROTID SLOWING												
EFFECT OF INSPIRATION												
EFFECT OF EXPIRATION												
EFFECT OF POSTURE	UNCHANGED	UNCHANGED	UNCHANGED	UNCHANGED	LEFT RECUMBENT POSITION—	SLIGHT	SLIGHT	0	MARKED VARIATION	SLIGHT	0	SLIGHT
RATE	100+	100+	100+	VARIABLE	SLOW	SLOW	RAPID	VARIES	VARIES	SLOW	SLOW	VARIES
FREQUENCY	LOW	LOW	LOW	HIGH	LOW	LOW	LOW	HIGH	HIGH	LOW	MEDIUM	MEDIUM
INTENSITY	+++	+++	++++	+++	+	+	+	+++	++	++++	+	++
QUALITY	DULL	DULL	DULL	SHARP SNAPPING	DULL	DULL	DULL	CLICKING	CLICKING	CLICK-LIKE	VALVULAR	VALVULAR
PALPABILITY	+++	+++	++++	0	0	0	0	0	0	0	+++	0
AREA BEST HEARD	APEX	APEX	APEX	SUPRAMAMMARY	APEX	APEX	APEX	PULMONARY	APEX	AORTIC	BASE	APEX
IMPORTANT ASSOCIATED CONDITIONS	C.H.F.	C. H. F.	C H F	M. S.	NONE	A-V BLOCK	A-V BLOCK	PULMONARY HYPERTENSION	NONE	NONE	B. B. B.	NONE

third heart sound is extremely rare in association with pure mitral stenosis.

Presystolic and fusion sounds occur in individuals with A-V conduction disturbances. In the presence of certain arrhythmias the presystolic sound may be accompanied by variations in the intensity of the normal first sound.

The pulmonary early systolic sound is loud, high pitched, and often has a sharp, clicking quality. It may give the impression of wide splitting of the first sound. Expiration accentuates the pulmonary systolic sound.

The so-called mid-systolic click has a characteristic quality and is prone to wander throughout systole and even into early diastole with changes in rate, respiration and posture.

The reduplicated sounds in bundle branch block are invariably distant and often more obvious on palpation. In the absence of heart failure, the rate is invariably slow. Audible splitting of the second sound rather than the first is the usual finding on auscultation.

SUMMARY

Carotid sinus pressure with consequent slowing of the heart rate caused the accessory sound in gallop rhythm to become first inaudible and then to disappear from the sound tracing in 85 per cent of thirteen cases reported by the authors. The validity of the technic as a method of differential diagnosis of triple rhythms has been affirmed through observations on numerous patients with various types of triple rhythm, eleven of whom are discussed. Whereas the true gallop sound invariably became inaudible when the ventricular rate slowed below an average "critical rate" of 91, other types of extra sounds became more obvious because of changes in timing, intensity or clarity. A classification of three-sound rhythms has been presented with a detailed acoustic, physiologic and clinical discussion of each. The pertinent clinical aspects of the differential diagnosis of triple rhythm have been summarized in tabular form.

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Pulmonary Stenosis and Interatrial Communication with Cyanosis*

Hemodynamic and Clinical Study of Ten Patients

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In patients who have pulmonary stenosis and atrial septal defect, blood may be shunted through the defect in either direction.[1–5] When the existing shunt is large and completely or predominantly right to left, the condition clinically may resemble the tetralogy of Fallot.[1,4–7]

It is the purpose of this paper to present clinical and hemodynamic data concerning a series of ten patients who had pulmonary stenosis and interatrial communication and were moderately or severely cyanotic. In all the patients the clinical findings indicated clearly the presence of pulmonary stenosis with an associated right-to-left shunt. It may not be possible to be certain of the location of the right-to-left shunt and in several of our patients a diagnosis of tetralogy of Fallot was considered most likely before cardiac catheterization.

In this paper the term "interatrial communication"[8] will be used to include a true defect located in any portion of the atrial septum, and also the condition of valve-competent patent foramen ovale which occurs in 25 per cent of normal persons. Under normal dynamic conditions the pressure in the left atrium exceeds that in the right atrium during a considerable portion if not all of the cardiac cycle and this difference is considered the basis for the left-to-right direction of the interatrial shunt in the usual uncomplicated case of atrial septal defect. In an individual with a valve-competent patent foramen ovale, this normal pressure gradient tends to close the potential interatrial communication and no left-to-right shunt occurs. When the pressure in the right atrium exceeds that in the left atrium, the shunt in atrial septal defect becomes partially or completely right to left. If for any reason the right atrial pressure is greater than that in the left atrium in a person who has valve-competent patent foramen ovale, the pressure gradient will tend to open this potential interatrial communication and cause a right-to-left shunt. Accurate localization of the site of the right-to-left shunt has been greatly facilitated by the introduction of indicator dilution technics. It is still, however, difficult or impossible to distinguish between valve-competent patent foramen ovale and valve-incompetent foramen ovale (atrial septal defect) by cardiac catheterization in cases in which the shunt is completely from right to left.

CASE REPORTS

The majority of the patients seen at the Mayo Clinic who have tetralogy of Fallot and in whom the pulmonary artery shadow is less prominent than normal have not been referred for cardiac catheterization up to the present time. This form of investigation is reserved for those in whom the clinical features are unusual or suggest another lesion. Some of the cases to be described were referred to the clinic with the diagnosis of tetralogy of Fallot.

The major clinical data in the present series of ten patients are summarized in Table 1. Roentgenograms of Cases I, III and X are reproduced in Figure 1 and electrocardiograms of these patients are reproduced in Figure 2.

CASE 1. The patient, aged twenty-one years, registered at the Mayo Clinic in February, 1954. He complained of severe effort dyspnea and fatigue, which had become progressively greater

* From the Sections of Medicine and Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

since 1952. He had been told by his parents that he was cyanotic at birth. His local physician referred him to the clinic for evaluation and possible cardiac surgery.

CASE II. The patient, aged nineteen years, registered at the clinic in August, 1952. She The patient sat down when tired but did not squat.

A chest roentgenogram disclosed slight enlargement of the cardiac silhouette and suggestive evidence of a right aortic arch. This was verified by fluoroscopy.

TABLE I

CLINICAL DATA IN TEN CASES OF PULMONARY STENOSIS AND INTERATRIAL COMMUNICATION

		Case									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Age (yr.)		21	19	5	13	5	22	3	18	23	23
Symptoms	Grade of cyanosis	3	1	2	1–2	2–3	3	2	2–3	2–3	1
	Cyanosis first noted	Birth	2 wk.; 7 yr.	Birth	Birth	Birth	Infancy	Infancy	Birth	Birth?	Childhood?
	Grade of clubbing	3	None	2	None	2	3	1	2	2	1–2
	Effort dyspnea and fatigue	Marked	Moderate?	Moderate	Present	Marked	Mild	Marked	Severe	Marked	Moderate
Auscultatory signs	Systolic murmur II–III space	Grade 2, coarse	Grade 3, rough	Grade 3	Grade 3	Grade 2	Grade 2, coarse	Grade 2–3, coarse	Grade 3, rough	Grade 3	Grade 3, rough
	Second sound	Not identified	Not identified	Single	Single	Single	Not identified	Single	Single	Not identified	Not identified
Radiologic appearance	Heart enlargement	R.V.+	Normal	Slight	Slight	R.V.+	Moderate	R.V.+	Normal	Slight	Slight
	Pulmonary artery shadow	Visualized+	Visualized+	Not visualized 0	Visualized+	Prominent ++	Not visualized 0	Prominent +++	Prominent ++	Prominent +++	Prominent ++++
	Vascular mark	Normal	Normal	Decreased	Normal	Normal	Slightly decreased	Decreased	Decreased	Normal	Normal

stated that cyanosis had been noted at two weeks of age associated with pneumonia. There had been no recurrence of cyanosis until the age of seven years. Since that time it had been persistent and associated with easy fatigability. There had been two recent transient episodes of paroxysmal tachycardia.

CASE III. The patient, aged five years, was registered at the clinic in February, 1954. The main complaints were shortness of breath, fatigue and palpitation on mild exertion. A cardiac murmur and cyanosis had been noted at birth, and cyanosis had been present since birth, exaggerated with crying and exertion.

CASE IV. The patient, aged thirteen years, registered on this admission in August, 1953, but had had prior examinations at the clinic in 1949 and 1950. A tentative diagnosis of pulmonary stenosis with ventricular septal defect had been made on the earlier admissions. The patient had been cyanotic at birth and had had persistent cyanosis since then, especially with effort. There were associated fatigability and effort dyspnea.

CASE V. The patient, aged five years, was registered at the clinic in October, 1952. He complained of marked effort dyspnea and fatigue. His mother stated that a few days after

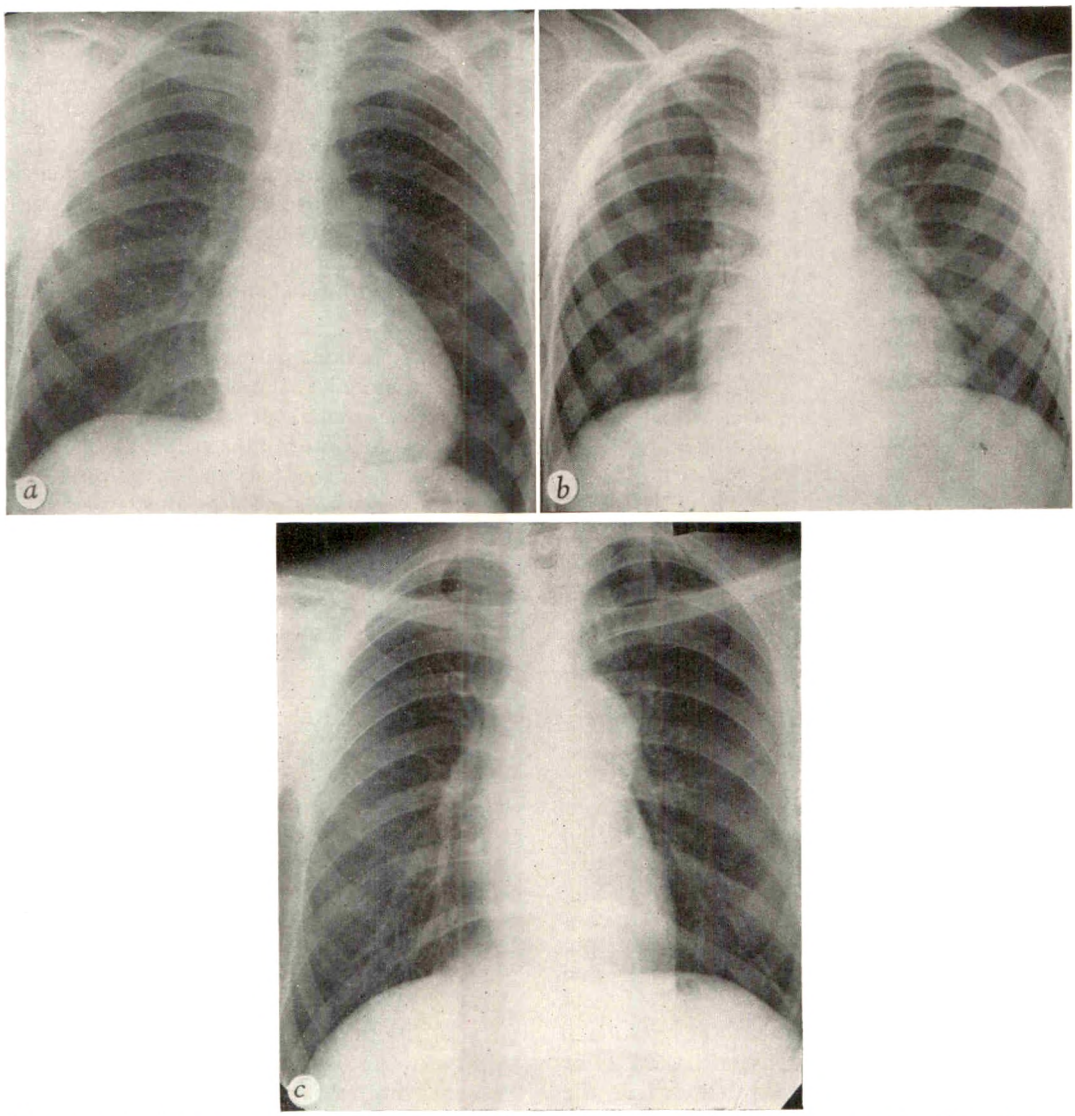


FIG. 1A to C. Postero-anterior roentgenograms of chest in Cases I, III and X. The presence of cardiac enlargement, the prominence of the pulmonary artery and the appearance of pulmonary vascular markings are given in Table I.

birth he had had an episode of unconsciousness and cyanosis when crying. During the second week of life he had two similar episodes. After this there were no further episodes of unconsciousness but cyanosis on crying and exertion was noted. A heart murmur was noted first at fifteen months of age by a pediatrician.

CASE VI. The patient, aged twenty-two years, registered at the clinic in July, 1952. He complained of mild effort fatigue and dyspnea. He had been told that cyanosis had been present since infancy and that he squatted in childhood when fatigued. He stated further that in childhood he could walk only twenty yards at one time, but that his exercise tolerance had improved with time and that he was now able to go up four flights of stairs without rest.

On examination the systemic blood pressure varied from 150 to 220 mm. of mercury systolic and from 115 to 170 diastolic. This was the only patient in the present series who had an elevated systemic arterial pressure. Urinalysis disclosed a specific gravity of 1.010, grade 2 albuminuria, grade 2 erythruria and hyaline and granular

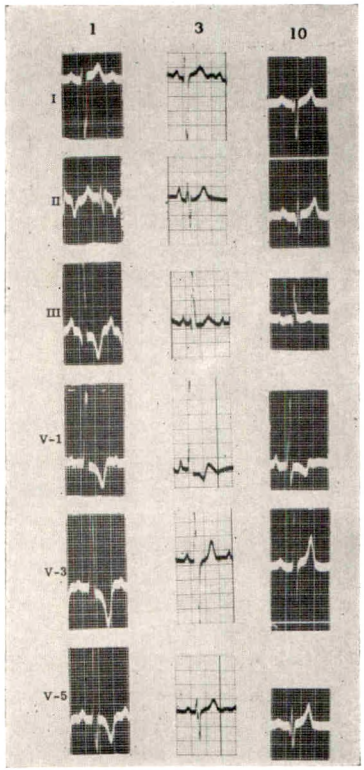


FIG. 2. Electrocardiograms for Cases I, III and X. The tracing for Case I was recorded at half the standard voltage. Note the moderate to severe right ventricular hypertrophy.

casts. The blood urea was 36 and 28 mg. per 100 cc. on two occasions. This patient died following operation. Sections of the kidneys revealed the presence of chronic glomerulonephritis with secondary hypertension. The improvement in exercise tolerance which the patient noted might be related to the development of systemic hypertension but the time of onset of the hypertension is not documented. There was moderate thoracic scoliosis.

CASE VII. The patient, aged three years, was registered at the clinic on this admission in April, 1952. A heart murmur had been noted at birth, and cyanosis on crying had been present in infancy. There had been progressive effort fatigue and dyspnea in the six months before the present examination. Squatting with fatigue was also present.

CASE VIII. The patient, aged eighteen years, registered at the clinic in November, 1950. She complained of effort dyspnea, fatigue and palpitation, which had been particularly severe since 1945. Cyanosis had been present at birth and clubbing had been noted in infancy.

CASE IX. The patient, a twenty-three year old man, registered at the clinic in April, 1953, desiring evaluation of his cardiac status. He was unable to obtain employment as a television repair man because of his cyanotic appearance and effort dyspnea. He had probably been cyanotic at or soon after birth but had not been significantly disabled until the age of seventeen years.

CASE X. The patient, aged twenty-three years, registered at the clinic in April, 1953. He planned on marriage in the near future and wished evaluation of his cardiac status. He was particularly interested in the possibility of cardiac surgery. He had noted easy fatigue, decreased exercise tolerance and cyanosis with moderate to severe exertion for as long as he could remember.

METHODS

The patients were studied by the cardiac catheterization technic, employing the method of Cournand and Ranges[9] as modified in apparatus and technic by Wood and associates.[10–12] Intracardiac and systemic arterial pressures were recorded using strain-gauge manometers.[13] The oxygen content of systemic arterial blood was determined by the method of Van Slyke and Neill,[14] and oxygen capacity was obtained manometrically after equilibration by the method of Sendroy[15] as modified by Roughton and associates.[16] For the recognition and localization of left-to-right intracardiac shunts, if present, multiple small samples of blood were obtained in the various chambers of the heart in rapid succession and the saturation of such samples was determined as they were drawn through a cuvette oximeter[17] attached via a three-way stopcock to the distal end of the cardiac catheter. Use of this instrument greatly facilitated the establishment of a certain diagnosis in many instances.

For the calculation of pulmonary and systemic blood flow Fick's principle[18] was used:

$$\dot{Q}_P = \frac{\dot{V}_{O_2}}{(C_{pv_{O_2}} - C_{pa_{O_2}})}; \quad \dot{Q}_S = \frac{\dot{V}_{O_2}}{(C_{sa_{O_2}} - C_{pa_{O_2}})}$$

Where $\dot{Q}_P$ = pulmonary flow, $\dot{Q}_S$ = systemic flow, $\dot{V}_{O_2}$ = oxygen uptake (L./min.), $C_{pv_{O_2}}$ = oxygen content (cc./L.) of pulmonary vein

blood,* $C_{pa_{O_2}}$ = oxygen content (cc./L.) of pulmonary artery blood, $C_{sa_{O_2}}$ = oxygen content (cc./L.) of systemic (radial) artery blood.

The right-to-left shunt (assuming no left-to-right shunt) was calculated from the relationship

$$\text{R-L shunt per cent} = \frac{\dot{Q}_s - \dot{Q}_p}{\dot{Q}_s} \times 100$$

expressed as a percentage of systemic flow.

In two patients satisfactory values for oxygen consumption were not obtained; in these the proportion of shunt was calculated on the basis of the oxygen saturation values according to the method of Burchell and Wood.[19]

Indicator dilution curves were recorded photographically, usually while the patient breathed 100 per cent oxygen, but at times while he breathed air, using cuvette and earpiece oximeters. Injections of T-1824 in doses appropriate to the size of the patient were made into the chambers of the heart and great vessels at different times during the catheterization procedure.[20]

HEMODYNAMIC FINDINGS

All of the patients (Table II) were found to have elevated right ventricular pressures. In six patients the right ventricular systolic pressure was greater than the radial artery pressure. In two patients these pressures were equal. In one patient the radial artery systolic pressure was greater than the right ventricular systolic pressure and in one patient, a child, systemic arterial pressure was not recorded.

The blood oxygen capacity was elevated in each patient. In four patients the values obtained were 30 volumes per 100 cc. or slightly higher.

Blood flow, expressed in liters per minute, was calculated in the pulmonary and systemic circulation in six patients. The pulmonary flow in each case was found to be less than the systemic flow. The right-to-left shunt was large in each patient. In five patients it was 50 per cent or more of systemic flow.

The diagnosis of pulmonary stenosis was made by withdrawing the catheter slowly from the pulmonary trunk to the right ventricle. (Fig. 3.) In two patients it was not possible to pass the catheter into the pulmonary trunk at the initial catheterization. In these patients, clearness of the lung fields in roentgenograms and the character of the auscultatory sounds in the pulmonary valve area militated against the diagnosis of pulmonary hypertension. Pulmonary trunk pressures subsequently obtained at operation confirmed that the diagnosis of pulmonary stenosis was correct.

No significant difference in the saturation of blood samples drawn from various positions on the right side of the heart through the cuvette oximeter was noted in any patient. This finding rendered the possibility of a left-to-right shunt unlikely.

Dye-dilution curves were recorded following injection of Evans blue dye (T-1824) at various sites in the heart and great vessels. In these patients the most significant dye-dilution curves were recorded following injection of dye into the right ventricle and at a site proximal to the right ventricle, either the right atrium or the venae cavae. In cyanotic patients, and especially when there is a right-to-left shunt at the atrial level, a variation in the oxygen saturation of systemic arterial blood frequently occurs. (Figs. 3 and 4.) This is usually related to the respiratory cycle and is probably due to a differing relative magnitude of shunt across the defect associated with changes in the intrathoracic pressure. It is of some practical importance that this variation in oxygen saturation may severely distort the dye-dilution curve recorded oximetrically. However, it is almost always possible to recognize the first appearance of dye with considerable accuracy. (Table III.) The dye-dilution curves recorded following injection into the right ventricle had normal or prolonged appearance time (Table III), indicating that the blood followed a pathway through the lungs and that no right-to-left shunt existed at ventricular level. The dye-dilution curves recorded following injection of dye proximal to the right ventricle had abnormally short appearance times and abnormal initial deflections, indicating that blood shunted from right to left through an interatrial communication. (See Figure 4.)

In Case IX, studied early in this series, the dilution curves were interpreted incorrectly. This patient was referred for cardiac catheterization with a diagnosis of pulmonary stenosis and atrial

* In this laboratory the oxygen content of pulmonary vein blood (cc./L.) for a patient breathing room air is taken to be: $C_{pv_{O_2}}$ = (98 per cent of oxygen capacity + 0.3) × 10.

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septal defect. When dye was injected into the right ventricle, a right-to-left shunt of 18 per cent was demonstrated but a larger right-to-left shunt (48 per cent) was found when dye was injected into the superior vena cava. These curves were taken to indicate the presence of evidence of tricuspid insufficiency was demonstrated in addition to an atrial septal defect. It was considered after these findings that the appearance of shunted blood following injection into the right ventricle was due to regurgitation of blood through the tricuspid orifice. In retrospect, more attention should have been paid to the greater magnitude of the shunt from the superior vena cava than from the right ventricle.

TABLE II

INTRACARDIAC PRESSURES, ARTERIAL OXYGEN SATURATION DATA, BLOOD FLOW AND SHUNT VALUES IN 10 CASES OF PULMONARY STENOSIS AND INTERATRIAL COMMUNICATION

Case No.	Age (yr.)	Sex	Pressures (mm. Hg)				Blood O_2 Capacity *	Arterial Oxygen Per cent Saturation (Van Slyke)		Blood Flow L./minute (air)		R-L Shunt † Per cent of Systemic Flow
								Patient Breathing				
			Right Atrium	Pulmonary Trunk	Right Ventricle	Radial Artery		Air	100 Per cent O_2	Pulmonary	Systemic	
I	21	M	15/8	6/4	200/17 to 240/15	118/78	30	66	74	1.6	4.2	62‡
II	19	F	13/2	22/6	157/10	109/68	22	86	93	4.6	8.2	44
III §	5	M	8/6	13/7	105/4	105/58	22	80	. . .	1.4	3.3	57
IV	13	F	7/2	12/8	129/11	128/70	22	90	99	3.3	4.5	25
V	5	M	10/5	. . . . .	205/10	. . . . .	32	63‖	76‖	. . .	. . .	Large¶
VI	22	M	13/8	. . . . .	197/14	156/113	31	70	79	. . .	. . .	57**
VII §	3	F	12/9	17/15	129/12	80/50	22	57	71	. . .	. . .	62**
VIII	18	F	9/4	11/8	196/9	121/87	23	79	. . .	. . .	. . .	44**
IX	23	M	7/4	8/3	187/7	109/71	34	79	88	1.8	4.0	55
X	23	M	10/4	10/3	91/8	124/64	23	86	98	3.8	5.8	35

* Volumes O_2 per 100 cc. blood.

† Shunt at atrial level.

‡ Flow, shunt and saturation values obtained while the catheter tip lay in the pulmonary artery (see legend to Figure 3).

§ Studied under anesthesia.

‖ Saturations by earpiece oximeter.

¶ From inspection of dye-dilution curves.

** Shunts calculated on the basis of oxygen-saturation data.

Pulmonary valvotomy was carried out by Dr. J. W. Kirklin in all except one of the patients. Case X was advised to undergo operation ventricular septal defect in spite of a considerable difference between the pressure in the right ventricle and the systemic arterial system. A pulmonary valvotomy was carried out but the patient subsequently died. At necropsy the ventricular septum was found to be intact but

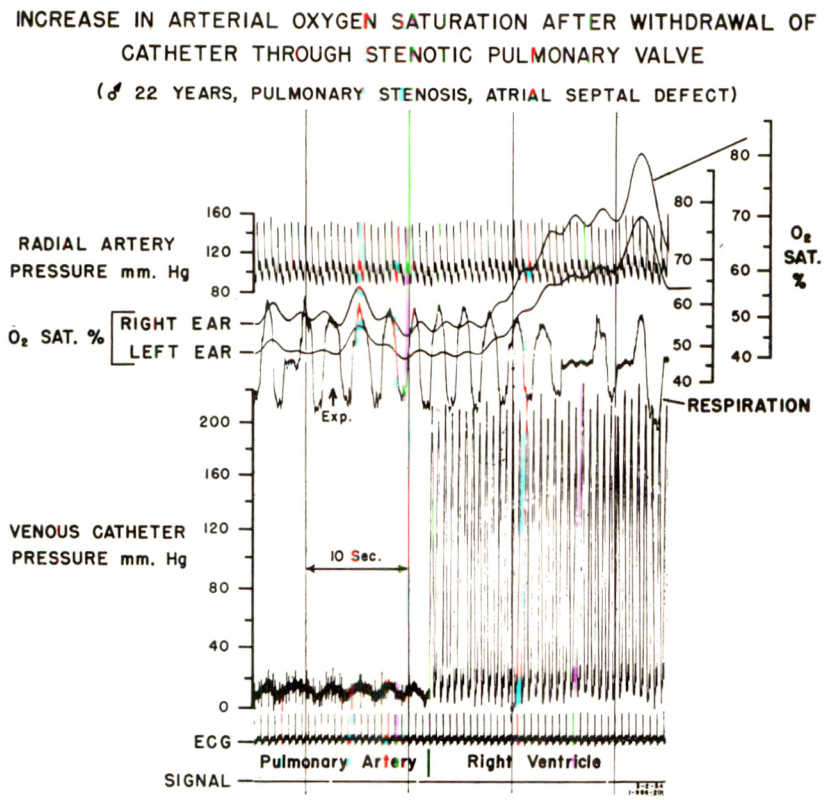


FIG. 3. Case I. Section of photographic record demonstrating the presence of pulmonary stenosis and the increase in oxygen saturation of systemic arterial blood consequent on the movement of a No. 6 catheter from the pulmonary trunk to the right ventricle while the patient was breathing room air. From above downward the record shows the right radial artery pressure, systemic arterial oxygen saturation as recorded by oximeters on right and left ears, the respiration, the pressure in the pulmonary trunk and in the outflow portion of the right ventricle, and the electrocardiogram. At the first break on the signal line the catheter was withdrawn slowly from the right pulmonary artery, and at the second signal it lay just above the pulmonary valve. Without further manipulation the catheter tip passed over a distance of less than 1 cm. into a zone of high pressure. Note the abrupt change in pressure, which indicates the presence of a valvular type of stenosis. The systemic arterial oxygen saturation as recorded by earpiece oximeters shows rhythmic fluctuations of the same frequency as respiration. When the catheter lay in the pulmonary artery, the arterial saturation ranged between 50 and 55 per cent. About six seconds after the catheter passed into the right ventricle the arterial oxygen saturation rose rapidly to approximately 70 per cent and the respirations slowed and became irregular. The systemic shunt and pulmonary flow values and the magnitude of the right-to-left shunt, all calculated while the patient breathed 100 per cent oxygen, were 1.8 L. per minute, 4.2 L. per minute and 57 per cent, respectively, when the catheter lay in the pulmonary trunk; and 2.4. L. per minute, 4.0 L. per minute and 40 per cent, respectively, when the catheter lay in the right ventricle. In this case and also in Case VII the size of the catheter (diameter 1.9 mm.) must have reduced the orifice of the pulmonary valve materially and increased the magnitude of the right-to-left shunt. In Case I, when breathing 100 per cent oxygen, the arterial saturations were 76 per cent, and 87 per cent by Van Slyke analysis when the catheter (diameter 1.9 mm.) lay in the pulmonary trunk and in the right ventricle.

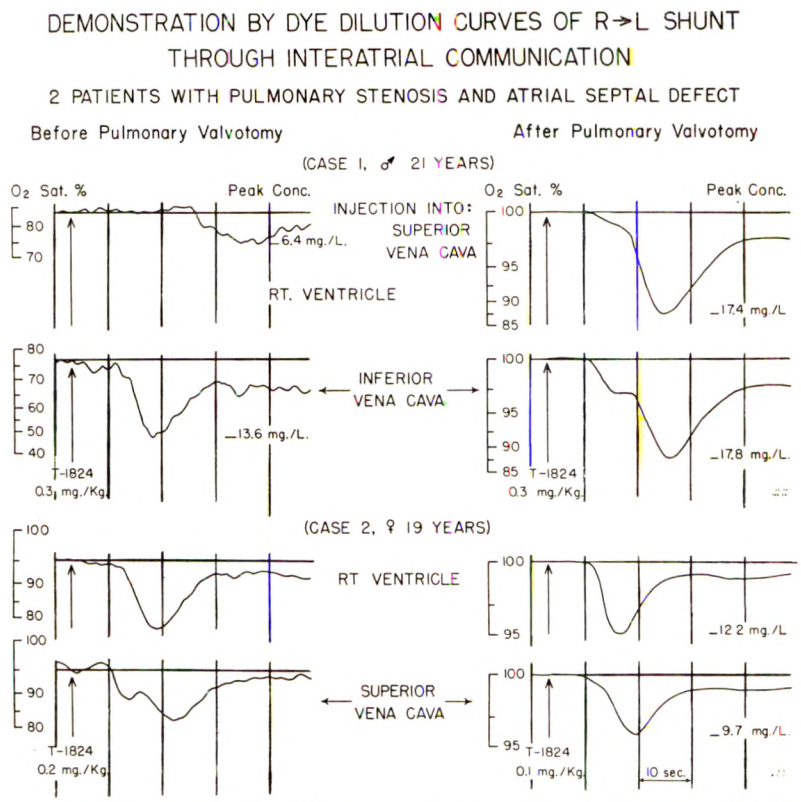


FIG. 4. Dilution curves recorded by earpiece oximeter following injection of T-1824 at the sites indicated in the center of the figure, before and after pulmonary valvotomy in Cases I and II. The oxygen saturation scales to the left of each curve are a relative index of the sensitivity of the oximeter used on the different occasions. The values for peak concentration of dye (to the right of each curve) were obtained from a dilution curve for radial artery blood which was recorded simultaneously by means of a cuvette oximeter. This instrument, but not at present the earpiece, can be calibrated in absolute values of dye concentration. The instant of injection and the amount of dye injected are indicated by the vertical arrow for each curve. The preoperative curves (to the left of the figure) show spontaneous fluctuations in oxygen saturation of considerable magnitude due to variations in the volume of the right-to-left shunt, which interfere with an accurate interpretation of the curves. Note, however, that in both patients the instant of first appearance of the dye is much earlier when dye is injected into the inferior or superior vena cava than when the injection is made into the right ventricle, this fact localizing the site of the right-to-left shunt to the atrial level. In both patients the curves are indicative of right-to-left shunts of large magnitudes. In the right panel are curves recorded following pulmonary valvotomy. Note the absence of spontaneous fluctuations in oxygen saturation, but in both patients an initial break still remains on the build-up portion of the dilution curve, indicating the presence of a small right-to-left shunt. Note also in Case I that the magnitude of shunt from the inferior vena cava exceeds that from the superior vena cava, a phenomenon frequently seen in cases with right-to-left shunt through an interatrial communication.

but he deferred treatment and died suddenly about one month after returning home. Cases VI, VII and IX died shortly after operation.[23] (Fig. 5.) Studies were carried out during the operation in all except one of the patients who underwent operation. These studies (Table IV) show a fall in right ventricular pressure following valvotomy. The findings in this series agree with those of Soulié and his associates[24] in that normal pressures are not usually obtained. As noted by Kirklin and associates[23] the results of these operative studies are not strictly comparable with the results of postoperative cardiac catheterization. The immediate postvalvotomy right

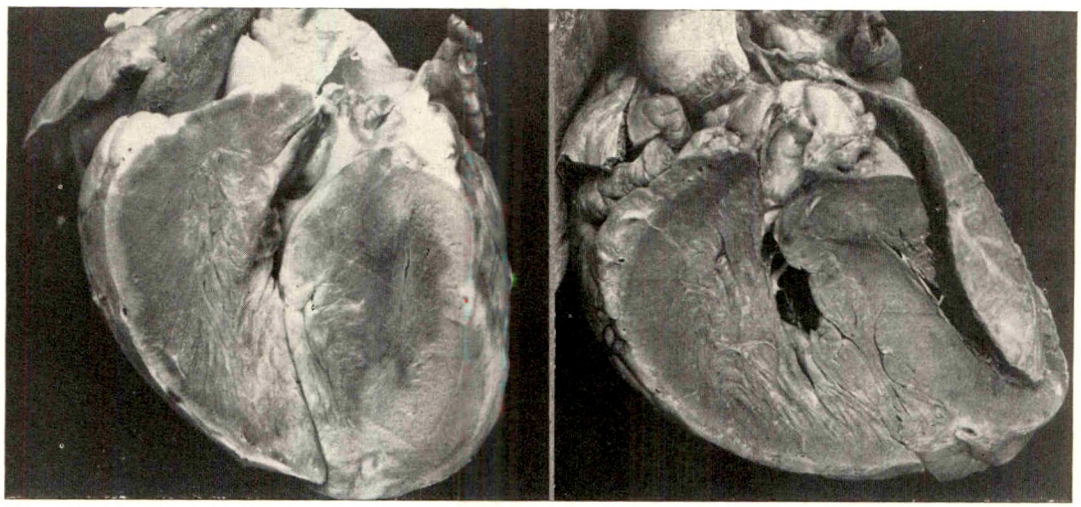


FIG. 5. The hypertrophied right ventricle and outflow tract of the hearts in Case VI (right) and Case VII (left). In Case VII a satisfactory pulmonary valvotomy had been accomplished but the patient succumbed to a respiratory infection (Case VI of Kirklin and associates[23]). This patient had a true atrial septal defect—valve-incompetent foramen ovale—but no left-to-right interatrial shunt was demonstrated at cardiac catheterization. Note in Case VI the unrelieved infundibular stenosis which was considered to be responsible for the patient's death. This patient had a valve-competent patent foramen ovale and no left-to-right shunt was possible. In this type of case indicator dilution curves allow a precise diagnosis to be made at cardiac catheterization.

ventricular pressures in Cases II and V are much greater than the pressures obtained at postoperative cardiac catheterization five months and one and one-half years later. In the four patients who underwent postoperative cardiac catheterization, reductions in right ventricular pressures of somewhat greater than 50 per cent were obtained. In one of the two patients studied postoperatively by earpiece oximetry, only arterial oxygen saturation was found to be increased. In Case IV the postoperative oximetry study did not show improvement in arterial oxygen saturation. However, she and the other patients who survived the operation noted marked subjective improvement.

TABLE III

TIME COMPONENTS AND MAGNITUDE OF R-L SHUNT CALCULATED FROM DYE-DILUTION CURVES AND DEGREE OF SPONTANEOUS FLUCTUATION IN OXYGEN SATURATION IN TEN PATIENTS WITH PULMONARY STENOSIS AND INTERATRIAL COMMUNICATION (RECORDED BY EARPIECE OXIMETER)

Case No.	Per cent Spontaneous Fluctuation*	Right Ventricle			Proximal Site		
		AT (sec.)	PCT (sec.)	R-L Shunt (%)	AT (sec.)	PCT (sec.)	R-L Shunt (%)
I	8.7	23.0	32.5	None	6.9	35	71
II	12.3	9.6	16.4	None	6.9	19.4	44
III	4.5	4.1	9.2	None	3.0	10.3	51
IV	7.0	7.3	14.7	None	5.5	17.0	20
V	4.6	15.2	23.0	None	5.9	30.5	49
VI	3.5	....	....	.....	5.0	21.9	58
VII	1.3	10.2	15.8	None	2.7	16.4	55
VIII	4.0	....	....	.....	8.5	14.2	62
IX	5.5	8.2	28.0	18†	6.0	32.0	48
X	7.4	10.9	18.4	None	6.7	20.8	41

* Spontaneous fluctuation in oxygen saturation associated with the respiratory cycle; difference between the maximal and minimal values for oxygen saturation per cent obtained from a representative portion of record.

Time components:[21]
AT = Appearance time.
PCT = Peak concentration time.
Per cent R-L shunt = R-L shunt calculated as per cent of systemic flow.[22]

† See text for comment.

COMMENT

Patients who have pulmonary stenosis and interatrial communication and who are cyanotic may resemble patients with tetralogy of Fallot in their clinical manifestations. Selzer and his group[1] and Johnson and Johnson[25] stated that pulmonary stenosis with interatrial communication is fairly common as a cause of cyanotic congenital heart disease, probably next in importance and frequency to tetralogy of Fallot among cases of cyanotic congenital heart disease with polycythemia and clubbing. In this laboratory fifty-one patients who had pulmonary stenosis and intracardiac shunt were studied by cardiac catheterization between 1947 and March, 1954. Seventeen (33 per cent) of these had interatrial communications and intact ventricular septa, of whom ten had arterial desaturation at rest. During this period a

number of patients with cyanotic congenital heart disease have undergone operation without prior cardiac catheterization, the diagnosis having been established on clinical grounds alone.

The entire group of patients with pulmonary stenosis and interatrial communication includes those with pure left-to-right shunt, who resemble patients with uncomplicated atrial septal defect clinically; those with a balanced shunt, who resemble patients with isolated pulmonary stenosis; and those with pure or predominant right-to-left shunt, who resemble patients with tetralogy of Fallot clinically. Some of the discrepant reports in medical literature might be related to this variation in clinical appearance which can be found in patients with the same anatomic abnormalities.

There now appear to be relatively few characteristics which set these patients clinically apart from patients with tetralogy of Fallot, although this opinion is not in agreement with that of Engle and Taussig.[26] McGregor and his group[4] and Joly and his group[5] have discussed the comparative findings in these two malformations. Breathlessness, weakness, cyanosis, cyanotic attacks, squatting and polycythemia were found in both conditions. Selzer and associates[1] indicated that pulmonary stenosis with patent foramen ovale occupied an intermediate place between the tetralogy of Fallot, the most cyanotic lesion, and the Eisenmenger complex, the least cyanotic of the three. They and McGregor doubted, however, whether the degree or time of onset of cyanosis was of any differential value in the individual case.

Our findings would be in general accord with the foregoing opinion and also with McGregor's statement that information about the age of

TABLE IV

INTRACARDIAC PRESSURES RECORDED AT THE TIME OF OPERATION BEFORE AND AFTER PULMONARY VALVOTOMY AND ARTERIAL OXYGEN SATURATIONS OBTAINED THREE WEEKS TO EIGHT MONTHS AFTER OPERATION

Case No.	Studies during Pulmonary Valvotomy							Postoperative Studies					
	Date of Operation	Pressures (mm. Hg)						Date of Study	Pressures (mm. Hg)		Arterial O_2 Saturation Per cent		R-L Shunt Per cent of Systemic Flow
		Before Valvotomy			After Valvotomy								
		Pulmonary Trunk	Right Ventricle	Right Radial Artery	Pulmonary Trunk	Right Ventricle	Right Radial Artery		Right Ventricle	Right Radial Artery	Room Air	100 Per cent O_2	
I	3/8/54	.....	60/17	43/38*	13/9	102/8	131/105	3/23/54	119/17	111/63	90	99.5	20†
II	8/18/52	14/8	125/5	100/70	13/8	110/5	85/65	1/12/53	57/15	110/71	92	99	14†
III	2/12/54	35/20	120/20	110/68	45/38	69/35	119/75	2/20/54‡	.....	.....	87	97	...
IV	9/14/53	28/15	165/5	78/62	27/14	75/5	98/62	10/9/53‡	.....	.....	84	95	...
V	11/18/52	23/12	.....	.....	25/13	122/18	.....	6/10/54	45/12	.....	91	97	15†
VI	7/24/52	34/27	220/33	130/107	28/19	188/18	157/115	§	.....	.....	....	....	...
VII	6/6/52	.....	162/17	.....	30/17	42/19	.....	§	.....	.....	....	....	...
VIII‖	.....	.....	.....	.....	.....	.....	.....	12/28/50	62/12	90/60	87.5	96.8	33
IX	5/15/53	17/11	135/10	70/40	17/10	60/10	70/42	§	.....	.....	....	....	...
X¶	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....	....	...

* During valvotomy patient had severe hypotensive reaction.
† Per cent shunt estimated from dye-dilution curves.
‡ Earpiece oximetry studies only.
§ Died following operation.
‖ Patient not studied during valvotomy.
¶ Patient deferred operation, died one month after study.
(Note: Cases I, III, VII, VIII, IX and X had valvular pulmonary stenosis only; Case IV had infundibular stenosis only; and Cases II, V and VI had combined valvular and infundibular stenosis.)

onset of symptoms may not be reliable. This is most likely to be true in adults, who may have only vague ideas about the exact time of onset of their symptoms and may have no information regarding possible cyanotic attacks before the onset of persistent cyanosis. In the present group of ten patients cyanosis was stated to have been present at birth or in infancy in eight. In one patient it probably began in infancy. In one patient persistent cyanosis developed at seven years but there was a history of transient cyanotic episodes in infancy.

The location and intensity of the murmur in these ten patients did not seem to be of value in differentiating them from patients with tetralogy of Fallot. The second sound in the pulmonary area was absent in five of these patients and single in the remaining five.

Roentgenographic evidence of reduced blood flow to the lungs is common to both conditions. Concavity in the region of the pulmonary artery segment is more characteristic of tetralogy of Fallot. In a series of twenty-five cases of tetralogy of Fallot confirmed at necropsy, however, Brinton and Campbell[27] found that a small heart with concavity in the area of the pulmonary artery segment had been noted in roentgenograms in only a few cases. They found some prominence in the area of the pulmonary artery segment in at least one patient, and considerable variation in heart size throughout the group. Normal chest x-rays have been noted in both conditions. We consider the roentgenographic findings of great importance in the clinical differentiation of these two groups of patients, despite the occasional case with tetralogy of Fallot with a prominent pulmonary artery and the infrequent case of pulmonary stenosis with interatrial communication in which the pulmonary artery is not well visualized. It is apparent from the pulmonary artery silhouettes in this group of patients (Fig. 1) that there may be considerable variation but the pulmonary artery was visualized in the routine postero-anterior chest roentgenogram in all but Cases III and VI.

The nature of the pulmonary stenosis, as determined at cardiac catheterization, is of interest in relation to the radiologic appearance of the pulmonary artery (Fig. 1) in our cases. In four of the five patients (Cases VII to X) in whom the pulmonary artery shadow appeared prominent, there was pure valvular stenosis. In the fifth patient (Case V) an associated short infundibular zone of stenosis could not be excluded. In three patients (Cases I, II, IV) the pulmonary artery shadow was visualized but was not prominent. One of these had valvular stenosis (Case I) but, in spite of the lack of prominence of the pulmonary artery shadow on the preoperative roentgenogram, definite post-stenotic dilatation of the pulmonary artery was noted at operation. Of the remaining two patients in this group an infundibular stenosis was thought to be present in Case IV and a combined valvular and infundibular stenosis in Case II. In two patients the pulmonary artery shadow was not visualized. In Case III the stenosis was valvular in type and in Case VI was combined infundibular and valvular. Case III had a right-sided aortic arch and in Case VI there was pronounced dorsal scoliosis. Either of these conditions might make visualization of the pulmonary artery shadow difficult.

Anomalies of the subclavian vessels and the right-sided aortic arches are seen frequently in cases of tetralogy of Fallot. Brinton and Campbell[27] found a right-sided aortic arch present in approximately one-quarter of their series of cases. Gordon and his group[28] pointed out that the presence of a right-sided aortic arch strongly suggests overriding of the aorta but noted that this anomaly had never been reported in a case with pulmonary stenosis, atrial septal defect and intact ventricular septum. McGregor and associates[4] state that Mannheimer[29] mentioned such a case but that no details were available.

In one of the cases in the present series (Case III) a right-sided aortic arch was found by x-ray. After catheterization it was not possible to exclude the presence of a ventricular septal defect in this patient, although there was no evidence of a shunt at the ventricular level. Indeed, the absence of a significant difference between right ventricular and systemic arterial pressure was consistent with an interventricular communication. (Table II.) At the time of operation, however, the right ventricular pressure exceeded the systemic pressure before valvotomy and fell considerably after valvotomy, while the systemic pressure actually increased. (Table IV.) These hemodynamic findings indicate that the ventricular septum was intact.

The electrocardiogram in cases of tetralogy of Fallot almost invariably discloses some right ventricular hypertrophy but not the extreme degree of right ventricular hypertrophy that has been reported in some cases of pulmonary

stenosis and interatrial communication with intact ventricular septum.[27] This feature may be of definite differential value, since an electrocardiogram showing evidence of very marked right ventricular hypertrophy would suggest a right ventricular pressure higher than systemic pressure, and hence would favor the inference of an intact ventricular septum. The electrocardiograms in all cases disclosed evidence of marked right ventricular hypertrophy. There were no instances of incomplete or complete right bundle-branch block.

Joly and his group[5] stated that the pulmonary stenosis found in association with an intact ventricular septum is characteristically valvular. In the cases in the present series, three were found to have infundibular or combined valvular and infundibular stenosis. Figure 5 (right) is a photograph of the heart from Case VI taken at necropsy showing combined valvular and infundibular stenosis. Figure 5 (left) is a photograph of the heart from Case VII, a case with purely valvular stenosis. Other pictures of these hearts have been published previously by Kirklin and associates.[23]

Use of currently available cardiac catheterization technics makes it possible to arrive at a correct diagnosis in patients with pulmonary stenosis and right-to-left shunt through an interatrial communication. The presence of pulmonary stenosis can be demonstrated when the catheter is passed through the pulmonary valve.[23] If the withdrawal of the catheter from the pulmonary artery through the pulmonary valve to the right ventricle is well controlled, it is frequently possible to identify a zone of intermediate pressure which indicates the presence of an infundibular stenosis. If no intermediate zone is found on slow and careful withdrawal, the stenosis is likely to be valvular but combined infundibular and valvular stenosis may be present in such instances.

The localization of intracardiac defects at cardiac catheterization depends either on the demonstration of arterialization in a cardiac chamber or on the successful manipulation of the catheter through the defect. When a pure right-to-left shunt exists, the site of the defect can be determined with certainty only if the catheter passes through it. Even then it is not possible to be certain that this defect is the site of the right-to-left shunt. In a case of combined atrial and ventricular septal defect with pulmonary stenosis and cyanosis studied recently in this laboratory, a left-to-right shunt was found at the ventricular level, while a right-to-left shunt at the atrial level was demonstrated by the dye-dilution method.[30]

The recent application of the technic of selective injections of T-1824 into the chambers of the heart and great vessels has been of great value in the solution of this problem.[20,31] Even small right-to-left atrial shunts in the presence of large left-to-right shunts have been recognized and quantitated by this method.[32] In seven of the ten patients in this series (Table III) selective dye injections indicated the site of the right-to-left shunt. In a number of these cases a certain diagnosis would not otherwise have been made.

The hemodynamic mechanisms responsible for the direction of interatrial shunts in the presence of pulmonary valve stenosis have not been completely elucidated. The size and nature of an interatrial communication may influence the direction of a shunt across it. Of more immediate importance, reversal of the usual interatrial pressure gradient has been demonstrated by Brecher and Opdyke[33] to be associated with right-to-left shunting of right atrial blood. The question remaining unanswered is the cause of this reversal of the interatrial pressure gradient. The patients included in the present study showed no evidence of cardiac failure, and records of the right atrial pressure did not indicate the presence of tricuspid insufficiency. The magnitude of the right ventricular pressure did not correlate with the magnitude of the shunt.

In patients who have pulmonary stenosis and interatrial communication the cardiac malformations are, at the present time, potentially curable. The pulmonary stenosis, if it is not a long infundibular tract, can be corrected. The atrial communication can be closed. Because such corrective surgery is available, many authorities have urged that these patients be treated by pulmonary valvotomy and not by a shunt operation. Gøtzsche[34] mentioned four patients with pulmonary stenosis and interatrial communication who were treated by shunt operations. In one of these patients congestive failure developed two years postoperatively. This patient was subsequently greatly improved by pulmonary valvotomy.

Brock and Campbell[35] mentioned three similar patients, children with pulmonary stenosis and intact ventricular septum who had undergone a shunt operation and who were

in a state of cardiac decompensation when Brock carried out pulmonary valvotomy. One patient died after operation but the remaining two subsequently underwent surgical repair of the artificial shunt and were markedly improved. All of the nine patients reported in this series who were treated surgically underwent pulmonary valvotomy. Two of these patients died in the postoperative period (Cases VI and IX, Kirklin and associates[23]). A further patient (Case IX of this series) died of intractable bleeding forty-eight hours after operation. The remaining six patients showed differing grades of improvement, but a residual right-to-left shunt was probably present in all cases. Certain individuals might be benefited if the interatrial communication were to be closed.

SUMMARY

1. Clinical and hemodynamic data have been presented in ten cases of pulmonary stenosis and interatrial communication with cyanosis. The clinical similarity between these patients and those with tetralogy of Fallot was noted.

2. Of the ten patients, cyanosis was stated to have been present in eight at birth or in infancy and it probably began in infancy in one other. In the remaining patient transient cyanotic episodes were noted in infancy and persistent cyanosis started at seven years of age.

3. Considerable variation was noted in the cardiac shadow on x-ray examination. The pulmonary artery shadow was visualized in eight of the ten patients on the routine anteroposterior roentgenogram of the chest. It was prominent in five patients.

4. Three of the patients had combined valvular and infundibular stenosis. Six had valvular stenosis only, and one had infundibular stenosis only.

5. One patient had a right-sided aortic arch. To our knowledge this is a very rare finding in a patient with pulmonary stenosis, interatrial communication and intact ventricular septum.

6. Systemic blood flow was greater than pulmonary flow in each case. In five patients the right-to-left shunt was 50 per cent or more of systemic flow.

7. The technic of selective injections of T-1824 was of great value in demonstrating the existence and localizing the site of the right-to-left shunts in the absence of any shunt from left to right.

Acknowledgment: The authors acknowledge the criticism and valuable suggestions offered by Drs. H. B. Burchell, J. E. Edwards and E. H. Wood.

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Hemodynamic Changes in the Bulbar Conjunctival Capillary Bed of Subjects with Hypertension Associated with "Cushing's Syndrome" or Pheochromocytoma*

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It has been established that individuals with hypertensive vascular disease display certain functional and morphologic changes in the capillary bed of the bulbar conjunctiva.[1,2] These are (1) vasoconstriction of the minute terminal arterioles and metarterioles; (2) increased reactivity of these vessels and the precapillary sphincters to topically applied epinephrine; (3) reduction in the velocity of peripheral blood flow and (4) a prominent change in vascular topography, namely, elongation, coiling and tortuosity of the true capillaries, particularly of their distal and venular portions, including the smaller collecting venules. These abnormalities do not all occur together in normal individuals and in patients with various normotensive diseases, but certain of the phenomena mentioned may be observed in a variety of conditions. For example, vasoconstriction was noted in subjects with rheumatoid arthritis and in cases of hyperglobulinemia, in these instances associated with increased metarteriolar reactivity to epinephrine. Approximately 8 per cent of normal individuals show minimal (Grade I to Grade II) elongation and coiling of the true capillaries. Thus no single observed peripheral vascular phenomenon can be described as "characteristic" of hypertensive vascular disease. It is only when all features observed in the hypertensive capillary bed were considered together that their unique association in this condition becomes apparent.

Within the past two-year period we have examined the conjunctival capillary bed in five cases of hypertension associated with Cushing's disease, three resulting from unilateral adrenal cortical adenomas and two from bilateral adrenal cortical hyperplasia. In addition, observations have been made in three hypertensive individuals proved by operation to have large functional pheochromocytomas. It is the purpose of this report to describe and compare the peripheral vascular changes of each entity; and to compare the findings with those previously noted in subjects with "essential" hypertensive vascular disease.

METHODS

As in previous reports, the bulbar conjunctiva of the individuals was examined with the standard type of slit-lamp microscope and slit-lamp illumination.[1] Subjects were seated before the instrument, and the head held relatively firmly in a combination chin-rest and forehead support, with gaze directed at a point light source flashing intermittently to facilitate fixation of the eye. After examining both the temporal and medial conjunctivas at magnifications of 47 × and 60 × in order to rule out conjunctival disease, the general anatomy and topography of the peripheral circulatory system was scrutinized. In most instances, photomicrographs were taken at 47 × with 35 mm. film. The over-all velocity of blood flow in the peripheral vessels was estimated in comparison with that observed in normotensive subjects without disease. The occurrence and degree of spontaneous vasomotor activity, as evidenced by speeding up and slowing of the blood column in the true capillaries, with narrowing and widening of the blood column at the precapillary region, was recorded in each individual.

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Because of the irregular changes in duration of the open and closed phases within capillary vessels, a subjective impression was obtained concerning the degree of vasomotor activity without attempting precise timing of the duration and frequency of the various phases in representative vessels. Reactivity to topically applied epinephrine was determined by applying solutions of various concentrations into the conjunctival sac, closing the patient's eye for a period of thirty seconds, then observing vessels previously studied for a period of one to two minutes. Beginning with concentrations of 1:800,000, progressively stronger solutions of 1:400,000, 1:200,000, etc., were employed at intervals of three to four minutes until a concentration was found at which "threshold" narrowing occurred in the selected precapillary vessel segments. As with the observations on the velocity of blood flow, this arbitrarily selected end-point has proven useful as a criterion for measurement of reactivity to a vasoconstrictor drug. In studies of over fifty subjects followed for a period of more than two years, the reactivity thus established has been found not to vary more than the standard error described previously.[2]

TABLE I

HYPERTENSIVE VASCULAR DISEASE

	Normal Subjects (125)	Essential Hypertension (100)	Normotensive Diseases (65)
General nature of peripheral blood flow	Vessels tonic; flow rapid and smooth, no stagnation	Vessels constricted; blood flow slowed, tissue ischemic	Highly variable
Spontaneous vasomotor activity	Grade 1–2 in 25% of cases	Grade 3–4 in 85% of subjects	Usually not seen
Velocity of capillary blood flow	Normal (0.026 mm./sec.)	Greatly reduced (0.011 mm./sec.)	Highly variable (0.004–0.03 mm./sec.)
Coiling, looping and tortuosity of capillaries and venules	Up to Grade 1 in 10% of cases	Grade 3–4 in 65% of cases	To grade 1–2 in 15% of cases
Epinephrine concentration required to produce threshold precapillary narrowing	1:37,000 (standard error, 1:3,700)	1:300,000 (standard error, 1:25,000)	1:315,000 (standard error, 1:32,000, highly variable)

RESULTS

The findings in subjects with hypertensive vascular disease previously described are summarized in Table I, and are compared there with those in normotensive subjects without disease and in normotensive subjects with various diseases. It is apparent that vasoconstriction with augmented spontaneous vasomotor activity, reduced velocity of peripheral blood flow in all peripheral vessels, and elongation, coiling and tortuosity of the capillaries and venules, occurred as an entity only in the subjects with hypertensive vascular disease. In Figure 1 the topographic plan of a normotensive subject without disease is shown. This may be compared to the pattern of the peripheral vascular system of a hypertensive subject without other disease shown in Figure 2. The difference in arrangement of the peripheral vessels is apparent.

Cushing's Syndrome. In Table II are the observations on subjects with Cushing's syndrome. The dysfunctional phenomena previously reported in hypertensive vascular disease are duplicated in subjects with hyperadrenalcorticalism. Figures 2 and 3 demonstrate that both groups also display similarly abnormal topographic arrangement of peripheral capillary vessels. There is elongation and coiling of the capillaries and venules, probably more noticeable in the venular portions of the individuals with Cushing's disease than in the hypertensive subjects without other disease states. The findings in the (conjunctival) capillary bed therefore do not permit separation of subjects with "essential" hypertensive vascular disease

from individuals whose hypertension is associated with adrenal cortical hyperfunction.

Pheochromocytoma. In Table III are the data on the patients with hypertension associated with pheochromocytoma. The terminal arterioles and precapillaries are narrowed and the tissues

It was possible in patient C. to observe the conjunctival capillary bed with the patient at rest and again during and following a hypertensive crisis produced by massage of the abdominal tumor. At resting blood pressure of 160/105 the capillary bed was ischemic, with

TABLE II
CUSHING'S DISEASE

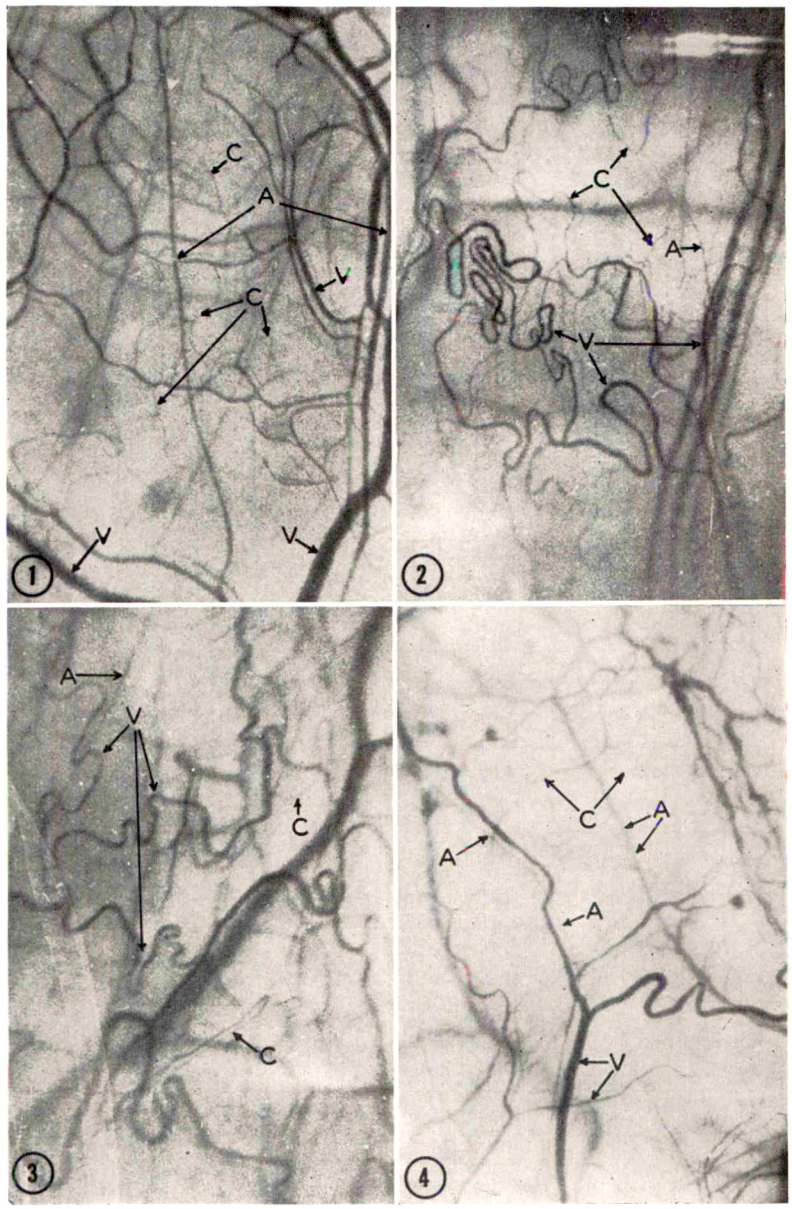
Patient	General Aspect	Topography	Epinephrine Reaction (× 1000)	Velocity of Flow
M. M.	Constriction of arterioles Grade 1, otherwise unremarkable	Grade 1 coiling and tortuosity of venular aspects of capillaries	1:200	Slightly reduced
Ch.	Grade 3 constriction with moderate ischemia	Grade 3–4 coiling and tortuosity of capillaries and venules	1:400	Greatly reduced
Lob.	Grade 3 constriction with moderate ischemia	Grade 3–4 coiling and tortuosity of capillaries and venules	1:400	Greatly reduced
Swe.	Grade 3 constriction and ischemia	Grade 3–4 coiling and tortuosity of venules and capillaries	1:300	Greatly reduced
Har.	Grade 3 constriction and ischemia	Grade 3–4 coiling and tortuosity of all small vessels	0:400	Greatly reduced

TABLE III
PHEOCHROMOCYTOMA

Patient	General Aspects	Topography	Epinephrine Reactions (× 1000)	Velocity of Flow
C.	Slight vasoconstriction	Normal, no abnormalities found	1:100	Normal
M.	Notable arteriolar narrowing, with ischemia	No abnormalities found	1:200	Slightly reduced
Mo.	Moderate amount of narrowing, with ischemia	No abnormalities found	1:200	Slightly reduced

are ischemic. The velocity of blood flow is reduced to approximately the levels at which it is noted in "essential" hypertensive vascular disease and in Cushing's syndrome. A striking difference, however, was the complete absence of capillary and venular elongation, coiling and tortuosity. (Fig. 4.) The anatomy and arrangement of elements in the peripheral vascular system of these patients were quite comparable to those observed in normotensive individuals, with or without any other disease.

moderately constricted arterioles and precapillary sphincters and epinephrine sensitivity of approximately four and a half times the normal range was observed. Vasomotion was minimal, the precapillary sphincters being predominantly narrowed. Within thirty seconds after beginning abdominal massage the blood pressure began to rise, attaining a level of 260/160 in sixty seconds. Coincidentally with the increase in blood pressure, the terminal arterioles and precapillaries were seen to



HUMAN CAPILLARY BED

FIG. 1. The normotensive subject shows absence of arteriolar constriction and relatively straight, patent capillaries and venules. (A, arterioles; C, true capillaries; V, venular portions of capillaries and collecting venules.)

FIG. 2. Essential vascular hypertension is accompanied by narrowed arterioles, fewer patent capillaries, and coiling, tortuosity and elongation of venular capillary segments and collecting venules.

FIG. 3. In Cushing's syndrome, the arterioles (in this case) were so narrowed that they are not readily noted in the photomicrographs. The capillaries and venules display prominent tortuosity and coiling, with elongation of the venular segments, very similar to that seen in essential hypertension.

FIG. 4. The capillary bed in pheochromocytoma has narrowed arterioles and few capillaries per unit of tissue; but the bizarre topographic abnormalities of Cushing's syndrome and essential hypertension are not observed.

constrict, almost to complete closure. At this time application of epinephrine of 1:800,000 produced complete closure of all of the patent arterioles. This represented a three to fourfold increase over the previously existing threshold level to twelve times the normal reactivity of arterioles to topically applied epinephrine. The blood pressure remained elevated and these findings persisted in the conjunctival capillary bed for approximately two and one-half minutes. The blood pressure then fell slowly within a period of four minutes to its former level of 160–165/105. During the fall in blood pressure the terminal arterioles gradually widened to resume their former partially constricted state, and blood flow increased to its previous speed. When the blood pressure had fallen to the preexisting level of 165/100, the reactivity of precapillaries to epinephrine returned to former levels of but four and a half times the normal value. It was assumed that the visualized vasoconstriction and ischemia were the result of an increase in the amount of epinephrine circulating within the blood stream. The augmented vascular reactivity to applied epinephrine probably represented a summation of the effects of the increased intravascular epinephrine with that applied to the outside of the arterioles and precapillaries.

Patient C. was explored surgically within a week after these observations and a large pheochromocytoma of the left adrenal gland was removed. The patient's blood pressure returned to normal levels. Observations of the conjunctival vessels seven days after operation and at least three times in the two-year interval following operation have demonstrated a normal topographic vascular arrangement and velocity of blood flow, with a reactivity of terminal arterioles and precapillary sphincters that is well within the usual range of normotensive individuals.

COMMENTS

It is apparent that the abnormalities present in the peripheral vessels (of the conjunctiva) in essential hypertension are similar to those present in like vessels of subjects with high blood pressure accompanying "Cushing's" disease.[2] In cases of pheochromocytoma, on the other hand, even though a persistent hypertension is present, the peripheral vessels in certain regards display a considerably different state. The arterioles and precapillaries are less hyperreactive to topically applied epinephrine, and there may be rapid variation in the degree of such vascular reactivity associated with the transient rise and fall in blood pressure produced by manual compression of the tumor mass (Patient C.). This latter picture contrasts sharply with the stability of epinephrine hyper-reactivity present in essential hypertension and in Cushing's disease.

A third and most outstanding difference noted was the complete absence in the patients with pheochromocytoma of the capillary coiling, tortuosity and elongation that were prominent features of the peripheral vascular bed of essential hypertensive and of hypertensives with Cushing's disease. The absence of this phenomenon in cases with adrenal medullary tumor, and its presence in hyperadrenal corticalism, suggests that development of such pathologic changes in the capillary bed may depend, at least in part, on excessive adrenal cortical activity.

The physiologic importance of capillary coiling, elongation and (in the venular portion) a widened capillary diameter is not clear. In view of the increased capacity which such changes afford in the single capillary, however, it is conceivable that the capillary bed *per se* might thus be enabled to hold considerably more blood. The increase in blood-holding capacity would reside primarily in the venular side of the peripheral vascular network, in contrast to diminished volume of the terminal arterioles resulting from arteriolar vasoconstriction. Thus the change may represent vascular adjustment to maintain the volume of blood within capillary bed elements. A second and equally plausible explanation of the increased capillary length and coiling may be based on arteriolar-capillary pressure relationships. Although the increased arterial blood pressure in hypertension is thought to be expended chiefly in the smaller arteries and the larger pulsatile arterioles, there is recent evidence that a portion of the increased pressure is transmitted peripherally to the metarterioles and their capillary offshoots.[3] With such an increase in the intracapillary pressure, conditions would greatly favor outward filtration of fluid and electrolytes, particularly in the proximal portion of the true capillaries. The accumulation of excess interstitial fluid as a result of such hydrostatic changes might be prevented by a corresponding increase in the factors which facilitate inward filtration of interstitial fluid, a

return to the intravascular space. The increase in length and in surface area afforded by the coiling and tortuosity (all of which occur chiefly in the distal portions of the capillaries where inward filtration probably predominates) may represent changes designed to increase greatly the potential ability to mobilize fluid from outside of the vascular bed. Thus although the rate of outward filtration at the proximal capillary segment might be theoretically increased, the expansion of surfaces for inward filtration in the more distal portions of the capillaries would maintain normal intravascular-extravascular fluid volume relationships.

CONCLUSIONS AND SUMMARY

1. The capillary bed in the bulbar conjunctiva was studied in eight patients with hypertension associated with pheochromocytoma or "Cushing's syndrome" (hyperadrenalcorticolism).

2. When compared with the findings in essential hypertension, the peripheral vascular system in hypertension caused by pheochromocytoma shows a similar but less notable vasoconstriction, less hyper-reactivity to epinephrine and complete absence of capillary and venular coiling, tortuosity and elongation. During a hypertensive crisis in one case, extreme increase in vasoconstriction, tissue ischemia and reactivity to epinephrine was observed, in contrast to the stability of the vascular status in essential hypertension.

3. Subjects with "Cushing's syndrome," on the other hand, display a pattern of vascular topography and response in the capillary bed that could not be differentiated from that found in essential hypertension.

4. The possible significance of these observations is discussed

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Kidney Biopsy in Acute Anuria*

With a Case of Acute, Bilateral Cortical Necrosis

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AMONG the various disorders of the kidney in which the kidney biopsy method has been employed, its usefulness has been demonstrated particularly in acute anuria. Evidence of this appears in Brun's findings based on kidney biopsies obtained in twelve patients with acute anuria (acute tubulo-interstitial nephritis).[3] The most essential histologic changes observed by Brun were: (1) The glomeruli were normal. (2) The proximal tubules presented low flattened epithelium and, in some cases, dilatation. (3) The distal tubules and the ascending part of Henle's loops were dilated, showing flattening of the epithelial cells. (4) In circumscribed areas the protoplasm of the epithelial cells in the proximal tubules sometimes showed so-called "hydropic" changes. Brun[3] also often found coexisting degenerative and regenerative (mitoses) processes in the tubular epithelium. (5) There were interstitial changes consisting of focal or diffuse edema with or without cellular infiltration. (6) Finally, the presence of hemoglobin-stained casts in the distal tubules, Henle's loops and collecting ducts, were noted. Tubular necroses were not observed. Brun has pointed out that interstitial changes play a much more important part than has been supposed by previous investigators. His findings also support the criticism of the term "lower nephron nephrosis" since, as pointed out by Oliver, MacDowell and Tracy,[19] among others, the infection is localized not only to the "lower nephron" but also, and perhaps chiefly, to the proximal tubules. The fact that postmortem autolytic processes are most pronounced in the proximal tubules may be the reason why the degenerative changes in the tubules have hitherto been underestimated.

As is known, tubulo-interstitial nephritis represents a very polymorphous group of diverse etiology; this also appears from Brun's series. In spite of this, it has not been possible to find histologic dissimilarities corresponding to the different etiologies.

In the three cases to be recorded the histologic findings were very different and, in at least two of the patients, were undoubtedly so characteristic of the underlying causes that a report of the cases might be of interest.

METHOD

The kidney biopsies were performed by the Iversen and Brun method,[12] and five biopsies in all were obtained in the three patients. Because of the presence of impaired renal function, the biopsies were not preceded by intravenous pyelography. Renal function was studied by twenty-four hour endogenous creatinine clearance and determinations of serum urea and serum creatinine. The kidney biopsies were uncomplicated. The authors had an opportunity to examine the sites of puncture at subsequent autopsies of two of the patients; nothing abnormal was observed.

CASE REPORTS

CASE 1. A thirty-three year old man, previously in good health, was assaulted by hooligans on August 20th, 1953, at 1 A.M. and incurred a bilateral fracture of the mandible. During the next three days he had headaches, nausea, dizziness and fever but was able to come in a taxi for treatment of the mandibular fractures.

Having become confused during the preceding night, he was admitted to the neurosurgical department on the morning of August 23 (three and a half days after the assault) in a completely unconsciousness state. On admission he was in shock. The pulse was impalpable, the

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action of the heart inaudible, the blood pressure 80 mm. Hg, and cyanosis of the lips and the finger tips was noted. Breathing was intermittent and gasping, and copious secretion in the respiratory tract was observed. In view of the possibility of an intracranial, extracerebral hemorrhage, surgery was performed, two holes being drilled on either side of the skull. No intracranial hemorrhage was found however, During the days that followed the patient was dull and semi-conscious, had respiratory rales and was cyanotic. On August 23 the output of urine was 250 ml., on August 24 (the day following surgery) it was 450 ml. During these twenty-four hours the patient was given 3000 ml. of fluid. The next day (August 25) the output of urine had fallen to 100 ml., the patient became increasingly restless, the blood urea level had risen, and acidosis and rising blood pressure were noted; further, atelectasis of the left lung was present. On August 25 the patient was given 50 ml. of a 50 per cent solution of sucrose, as cerebral edema was suspected.

On August 26 the patient was transferred to Department III (Medicine) owing to anuria, uremia and arterial hypertension. He was dull and semi-conscious on admission, though he reacted when spoken to and gave adequate replies. However, he was not oriented in time or place. There was some edema, especially of the legs. The blood pressure was 190/135 mm. Hg; serum urea, 244 mg. per 100 ml.; and serum creatinine, 10.4 mg. per 100 ml. Catheterization yielded only 20 ml. of slightly bloody urine.

At 5 P.M. on the day of admission the patient had an attack of generalized convulsions for about fifteen minutes and then lost consciousness. Ophthalmoscopy showed normal eyegrounds. A neurologist considered that cerebral edema was present and recommended the administration of sucrose. Therefore, for the second time in forty-eight hours the patient was given 50 ml. of a 50 per cent solution of sucrose intravenously.

The next day (August 27) the serum urea had risen to 354 mg. per 100 ml., the serum creatinine to 14.6 mg. per 100 ml., and the oliguria persisted. At 1:30 P.M., that is, after four days in a state of shock, he died; the principal cause of death was presumed to be cerebral edema.

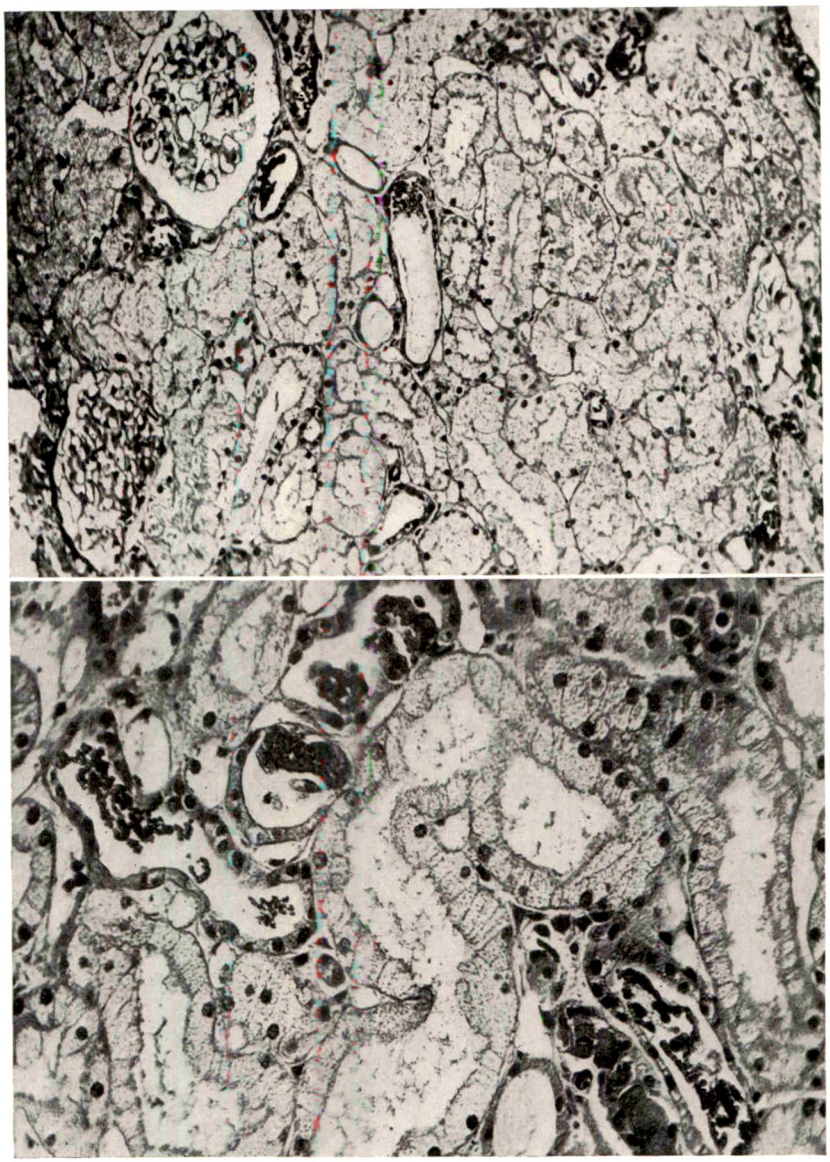
Kidney biopsy had been performed one hour and forty minutes prior to death. The tissue obtained measured about 9 mm. in length (Figs. 1 and 2) and consisted of cortex containing 10 glomeruli, all of which were normal. The epithelium in all proximal tubules and in many distal tubules had undergone pronounced "hydropic" change. In many tubules there were either no nuclei or very few; many of the nuclei present were pycnotic. The cells in the proximal tubules were rather tall, and the lumina were slightly dilated in many areas. As already mentioned, the epithelium in a number of distal tubules showed similar changes, in others it was flattened. A few slightly hemoglobin-stained casts were seen in the lumina of the distal tubules. No inflammatory infiltration, no interstitial edema and no vascular changes were seen. The histological diagnosis was diffuse "hydropic degeneration" and some hemoglobin-stained casts.

Autopsy showed: (1) Three fractures of the mandible had been sustained. (2) Hematomas were present in the pharynx and the larynx and in the subcutaneous tissue on the anterior surface of the neck. (3) Severe edema and pronounced hyperemia of the brain were noted. (4) The kidneys were of normal size with smooth surfaces and normal proportions of cortex and medulla; the cortices were somewhat pale. (5) The lungs showed congestion and edema, and atelectasis of the left lower lobe was present.

In addition to the same changes as were seen in the biopsy, histologic examination of the kidneys at necropsy revealed scattered small interstitial infiltrates of lymphocytes and macrophages in the cortex, which also showed distinct interstitial edema in some areas. Considerable amounts of hemoglobin-stained casts were present in Henle's loops and in the collecting tubules.

Summary and comments: A thirty-three year old man developed deep shock, anuria, uremia, hypertension and edema three days after severe head trauma and died on the seventh day. The principal cause of death was presumed to be cerebral edema. Kidney biopsy and autopsy specimens showed slight acute tubulo-interstitial nephritis and pronounced diffuse "hydropic" tubular changes, especially in the proximal tubules. The latter lesion was probably due to intravenous administration of sucrose.

There is every reason to believe that this patient was overhydrated; this undoubtedly contributed to his death. The autopsy revealed both cerebral and pulmonary edema (both of which, however, could have been due to uremia; the brain edema could have been possibly due also



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FIG. 1. Kidney biopsy with normal glomeruli, pronounced "hydropic" changes in all proximal tubules (probably due to intravenous sucrose administration) and a few hemoglobin-stained casts in the distal tubuli with flattened epithelium; × 145. (Case 1.)

FIG. 2. Kidney biopsy from the same preparation as Figure 1 showing the same lesions; × 280. (Case 1.)

to the trauma) and hypertension had been present.

The significance of the diffuse so-called "hydropic" changes found in the tubules in this case is not known. They resemble a diffuse form of the change which Brun[3] and others have found in acute, tubulo-interstitial nephritis in more focal areas. Whether it can be considered indicative of an intracellular edema cannot be stated with certainty.

The patient had been given two intravenous injections of sucrose while the renal function was much impaired and oliguria was present. It is known from the literature[1,6,8,10,11,15,16] that, when given intravenously to dehydrate the tissues, especially the brain, sucrose may pro-

duce marked "hydropic" changes in the proximal tubules of the kidney; however, the significance of these changes is not known. With regard to the effect on the renal function of a single injection of sucrose, opinions differ. Joliffe, Shannon and Smith[13] have shown that sucrose injected subcutaneously does not alter the xylose or the urea clearances, whereas it has been shown by Helmholz[10] and Helmholz and Bollmann[11] that the excretion of phenolsulfonphthalein decreases after a single injection. But it does not appear that repeated injections of sucrose in physiologic quantities can give rise to permanent renal lesions.[8,10,11] It is not known whether sucrose may injure tubular cells in a kidney which does not function normally, but it would seem irrational to use sucrose for dehydration in acute oliguria when the organism is unable to eliminate water.

With regard to the renal changes in the present case, they must be considered to have been caused chiefly by the treatment with sucrose and to a lesser degree by acute tubulo-interstitial nephritis (hemoglobin-stained casts and interstitial infiltrates).

Case II. A thirty-five year old woman had been feeling tired and nervous during the past year and a half. In March 1953, she had an infection considered to be pneumonia. Since that time the sedimentation rate had been increasing, and she had become progressively fatigued, nervous and irritable.

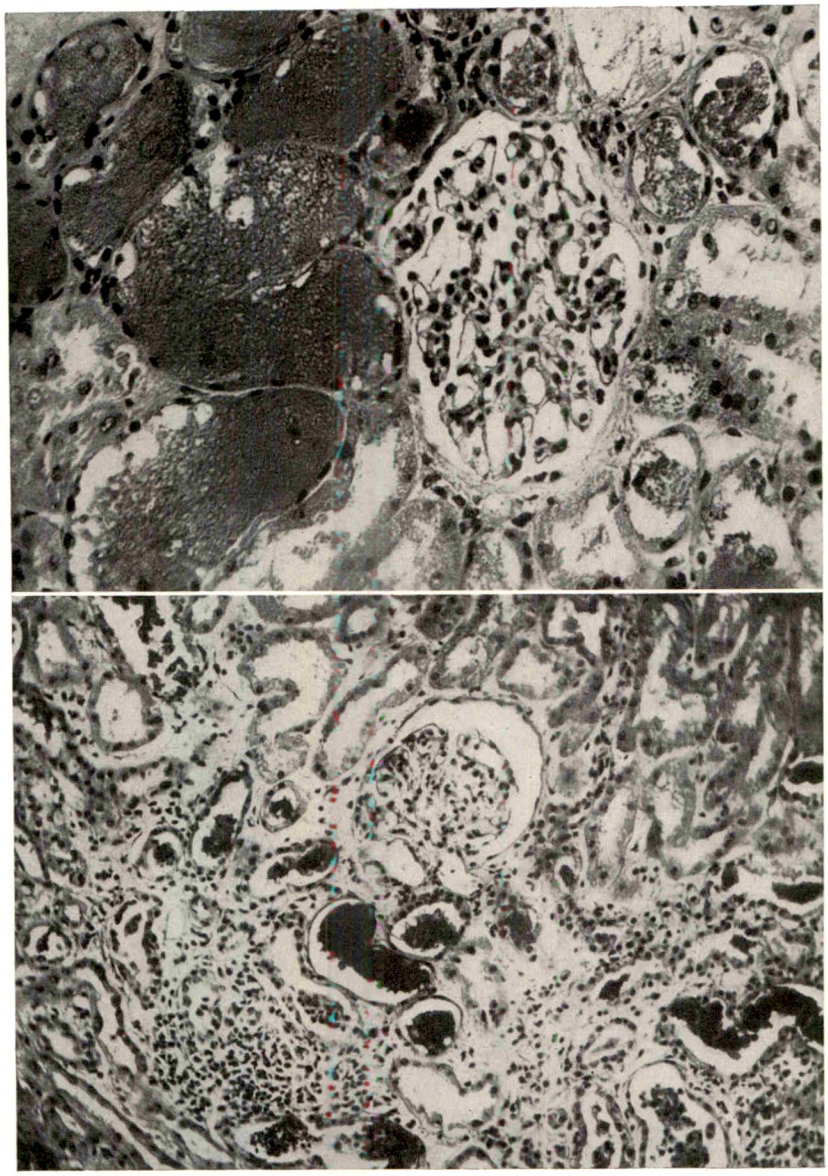
Early in the night of August 18, 1953, she suddenly became ill, feeling very sick with chills, copious diarrheal discharges, profuse vomiting, nausea, headache and lumbar pain radiating into the groin. There was slight vaginal bleeding. She had a fever of 40°C. Four hours after onset she was admitted to Surgical Department I, Kommunehospitalet, for observation for febrile abortion. Gynecologic examination showed that the uterus was enlarged, corresponding to a pregnancy in the eighth week, and there was moderate bleeding. She had not menstruated for two months.

A good deal of medicine had been taken at home, none of which was known to produce hemolysis. When questioned several times both the patient and her husband denied that abortifacients had been taken; their denial seemed trustworthy. Questioning revealed that no one else in their home had become sick following supper.

Catheterization on the morning of August 18 yielded 100 ml. of bloody urine. On examination at 12 A.M. she was neither exhausted nor in shock but had a brownish, icteric, rather mulatto-colored appearance. At this early stage of the disease she already had severe anemia (hemoglobin percentage, 49), and the serum was of a dark reddish brown color (hemoglobin percentage in serum, 11). Marked leukocytosis was present in the peripheral blood (64,000). The blood was examined to determine the cause of the hemolysis but no signs were found to indicate the presence of immune-hemolytic anemia. On the same day the patient was transferred to Medical Department III for uremia (serum urea, 135 mg. per 100 ml.; serum creatinine, 4.9 mg. per 100 ml.).

Kidney biopsy was performed twice on this patient, on the third day of the disease (August 20) and on the ninth day (August 26). Pronounced oliguria was present on both occasions. The specimen of renal tissue obtained on August 20 measured 3 mm. in length (Fig. 3) and consisted of cortex containing three glomeruli in all; these were normal. The proximal convoluted tubules appeared to be normal also. However, the epithelium was comparatively low. and in a few areas the nuclei apparently protruded directly into the lumina which appeared rather wide but hardly dilated. In almost all distal tubules there were coarse grained, distinctly hemoglobin-stained casts which apparently distended the lumina, the epithelium thus being flattened. No inflammatory infiltration was noted, and there seemed to be no interstitial edema. The histological diagnosis was numerous hemoglobin-stained casts in the distal tubules.

The biopsy specimen of the kidney, obtained on August 26, measured about 3 mm. in length (Fig. 4) and consisted of cortex containing four glomeruli, all of which were normal. The epithelium was consistently low in the proximal tubules and very low in the distal tubules; most of the latter had deeply hemoglobin-stained casts which contained remnants of nuclei in some places. There was no definite hydropic change, no tubular necrosis. There were several small interstitial inflammatory infiltrates consisting of lymphocytes and macrophages, very few neutrophil leukocytes and a few plasma cells. Slight interstitial edema was observed in some areas. No vascular changes were noted. The histologic diagnosis was acute tubulo-interstitial nephritis.



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FIG. 3. Kidney biopsy with normal glomerulus and distal tubuli distended by hemoglobin-stained casts; × 280. (Case II, third day after hemolysis.)

FIG. 4. Kidney biopsy with the same lesions as those shown in Figure 3; edema and slight inflammatory infiltration interstitially; × 145. (Case II, ninth day after hemolysis.)

Chart 1 shows how the oliguria varied from 67 to 122 ml./24 hours for a fortnight; only then did the output of urine begin to increase spontaneously. The urine was dark brown during the first six days of the disease. During the period of oliguria the serum urea rose progressively, the maximum being 468 mg. per 100 ml. on the sixteenth day. The twenty-four hour creatinine clearance was measured daily and was estimated to be 0.2 to 0.7 ml./minute during the period of oliguria. The patient remained conscious, and vomited a good deal. On the eleventh day of illness, as the oliguria persisted, the patient was transferred to the University Medical Clinic in Lund, Sweden (N. A. Alwall) for treatment by dialysis. However, the output of urine began to increase spontaneously from the thirteenth to the fourteenth day of the disease

and slowly rose to very high levels. From the sixteenth day the serum urea began to fall. As the patient's general condition never became serious, it was considered in Lund that hemodialysis was not indicated. On the twenty-fourth day of the disease she was transferred back to

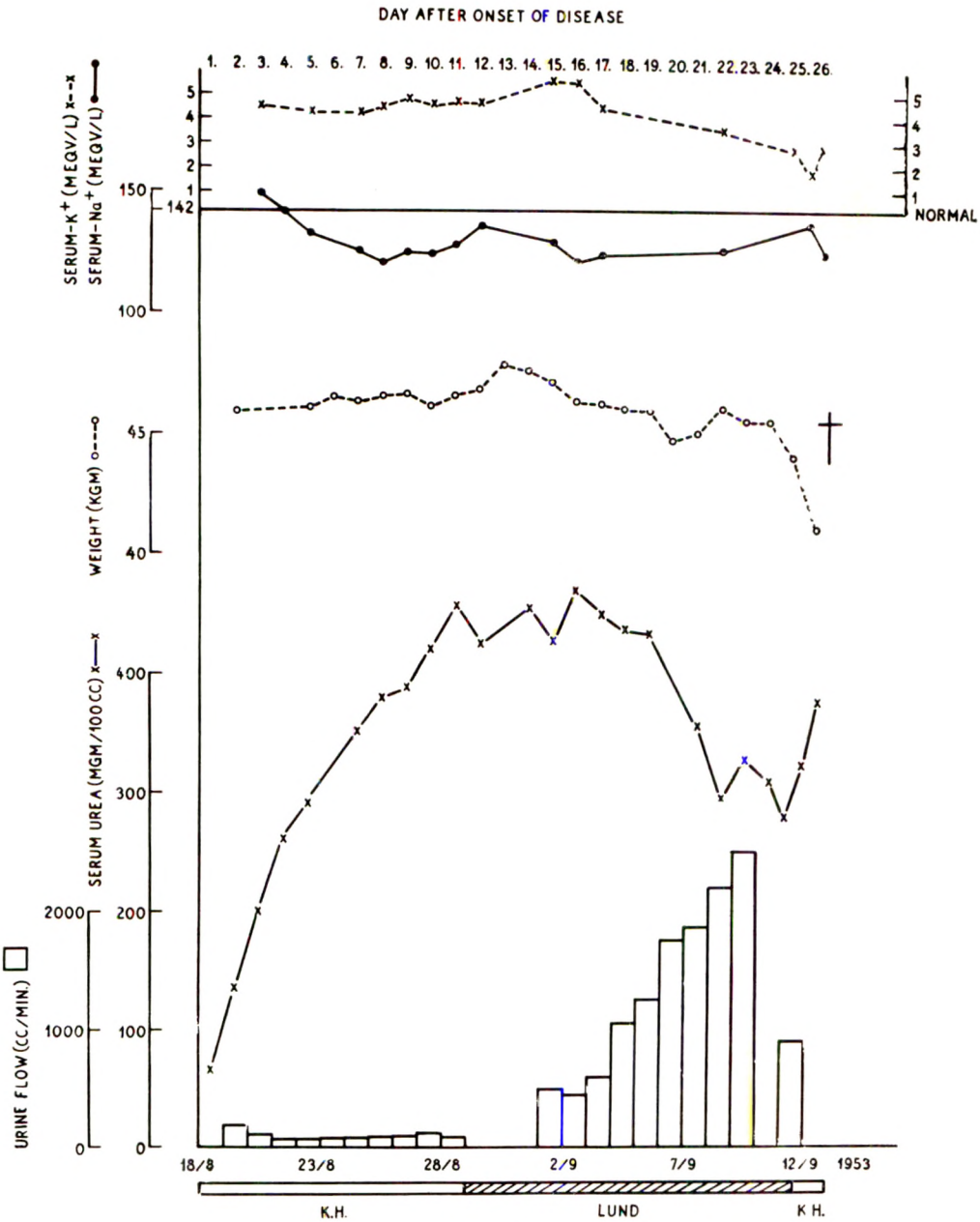


Chart 1

Copenhagen. On her return she had developed stomatitis and large conjunctival hemorrhages but was feeling well; towards evening she became restless and anxious, was slightly obtunded, and the pulse was bigeminal. Examination of the blood revealed hypokalemia (serum potassium, 2.7 mEq./L.). Next day (the twenty-fifth day of the disease) an attempt was made to remedy the hypokalemia by the administration of potassium by mouth and by infusion. As the serum sodium (111 mEq./L.) and the serum chloride (91 mEq./L.) showed low values, the patient was

also given a 0.9 per cent saline solution. In the afternoon she developed faint muscular twitchings and at 7 P.M. developed convulsive seizures of the right arm and the right side of the face. Three hours later she died of pulmonary edema.

Autopsy revealed a subarachnoid hemorrhage around the left hemisphere and a small area of encephalomalacia in the left capsula interna. Pronounced acute congestion and edema of the lungs was also noted. The uterus revealed a small intumescence in the mucous membrane, surrounded by hemorrhages; histologic examination revealed decidual tissue, indicating that the patient must have been pregnant. Histologic examination of renal tissue showed numerous hemoglobin-stained casts in the distal tubules, Henle's loops and collecting ducts.

Summary and comments: A thirty-five year old pregnant woman suffered from severe (toxic?) hemolytic anemia with renal failure and oliguria-anuria of thirteen days' duration. The renal function improved during conservative treatment but the patient died on the twenty-sixth day of subarachnoid hemorrhage and localized encephalomalacia. Kidney biopsy and postmortem specimens showed numerous hemoglobin-stained casts in almost all distal tubules.

In view of the sections obtained by kidney biopsy (Figs. 3 and 4), one must consider whether the numerous hemoglobin casts found played a part in the causation of oliguria by blocking the tubules; as already mentioned, both biopsies were performed during the period of oliguria. Later, the patient had a phase of fairly pronounced polyuria. Autopsy specimens of renal tissue still showed many of the casts. This fact suggests that the casts need not necessarily play a part in the oliguria, as one might be inclined to believe.

CASE III. This was a twenty-one year old woman who had been previously in good health. As the result of a local trauma six months before admission, she had a stillborn child about two months before term. Two months before admission the patient had an early abortion. She was not pregnant at the onset of the present disease. Except for an attack of pyelonephritis of short duration, treated at home a year and a half before admission, she had never had symptoms of infection of the kidney.

The patient was admitted to Department III, Kommunehospitalet, on October 13, 1953 because she had not passed urine for four days. The disease had begun rather suddenly on October 8 with bilateral lumbar pain, nausea, vomiting and rise of temperature to about 38°C. There had been no preceding disease, no dysuria, no edema, no disturbances of vision. The patient had not been in contact with toxic substances, had not taken any drugs which might produce renal damage, and there were no signs of hemolysis in the blood.

She was conscious and was not exhausted on admission. Except for moderate bilateral tenderness in the lumbar regions, results of physical examination were normal. Blood pressure was 115/60 mm. Hg. Immediately after admission 20 ml. of slightly turbid urine with a specific gravity of 1.020 was evacuated by catheterization. Microscopic examination of the urine showed a number of erythrocytes, a few leukocytes, epithelial cells and hyaline casts. The patient produced no urine for the next sixteen hours. Cystoscopy sixteen hours later did not show any urine in the bladder. Moderate cystitis was present, and no urine was obtained by ureteral catheterization. No obstruction was found, and direct pyelography revealed nothing pertinent.

On October 14 (the sixth day of the disease) the serum urea was 253 mg. per 100 ml., and the serum creatinine was 12.4 mg. per 100 ml. Serum chloride was 83.3 mEq./L., serum sodium 121 mEq./L. An electrocardiogram showed inverted T waves but laboratory investigations gave otherwise normal results.

Kidney biopsy was performed twice, on the seventh and eleventh days of the disease (October 15 and 19, respectively). The output of urine was low on both days. (Chart II.) The kidney biopsy obtained October 15 consisted of two small tissue cylinders measuring 3 and 5 mm. in length, respectively, the smaller one consisting of cortex, the longer one of medulla. Most of the cortical tissue was necrotic; the interstitium was edematous, in parts markedly infiltrated with blood, and extensively infiltrated with leukocytes. One could discern the shadowy markings of tubules (Fig. 5) in which no nuclei could be observed. However, one end of the cortex specimen contained three glomeruli which were fairly well preserved. The basement membranes showed slightly diffuse hyaline thickening and some contained small hyaline precipitates. The tubules in the circumference of these glomeruli showed fairly marked necrobiotic change, and most of these tubules contained large, hyaline casts which filled the highly dilated lumina.

The medullary tissue showed no necrosis or necrobiosis, and scarcely any increase in interstitial connective tissue was noted. There was no inflammatory infiltration in the medulla. Considerable amounts of hyaline or slightly hemoglobin-stained casts were present in the loops and the collecting ducts, but there were no coarsely granulated, deeply hemoglobin-stained casts like those present in tubulo-interstitial nephritis.

The partly necrotic cortical tissue seemed to resemble renal tissue from an ischemic infarct or from the hyperemic border zone of such an infarct. The histologic diagnosis was partly necrotic cortical tissue—tissue from a kidney infarct (?)—medullary tissue with a number of hyaline and slightly hemoglobin-stained casts but without other changes.

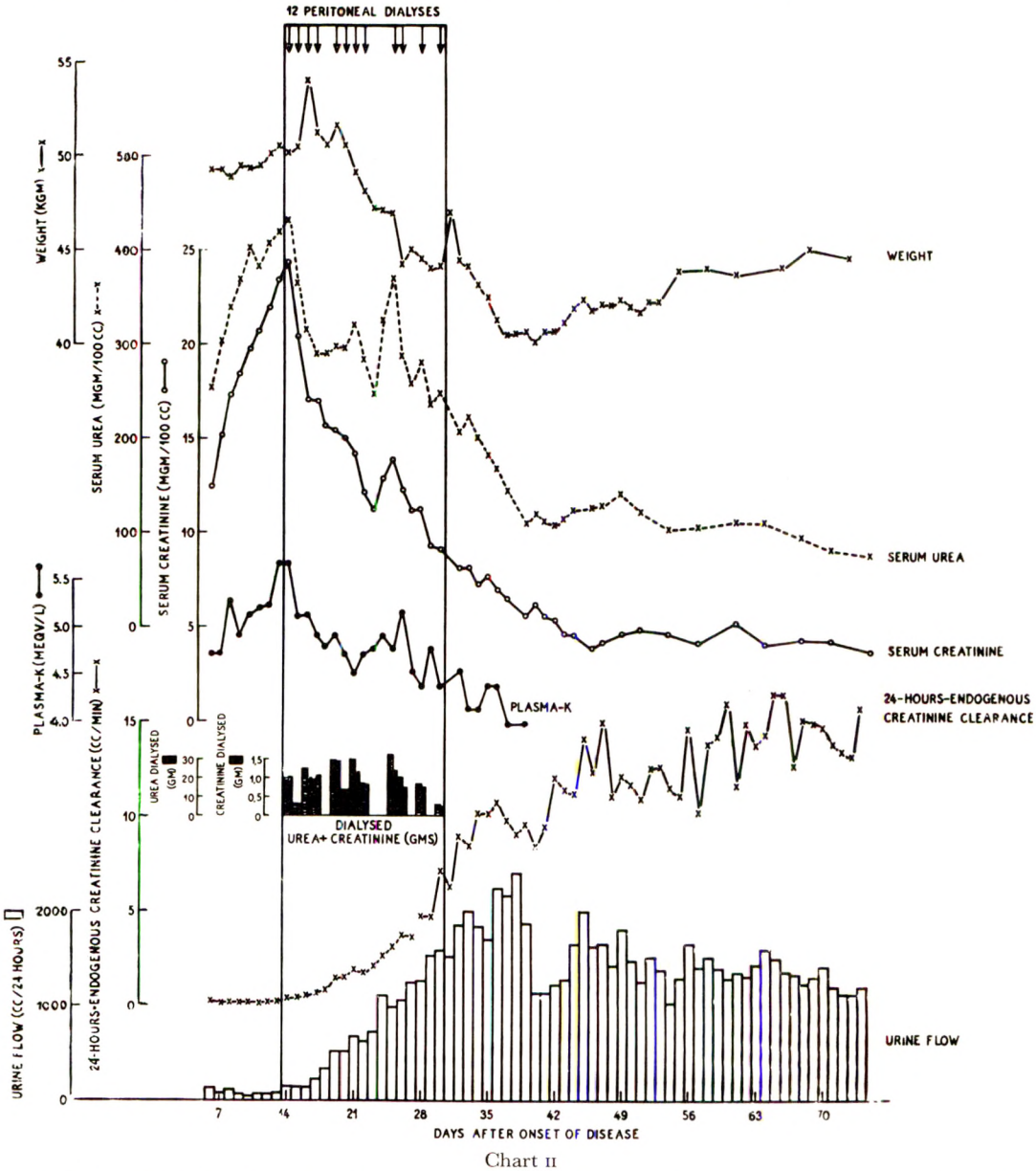
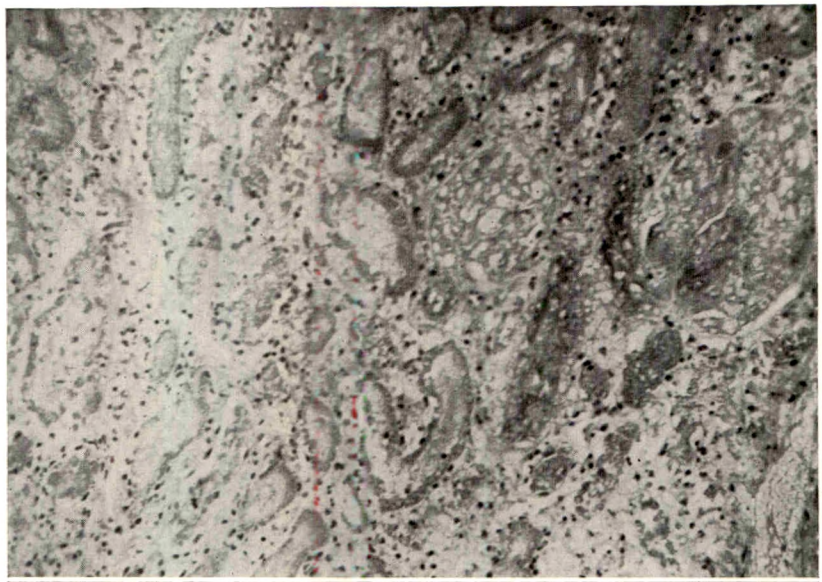
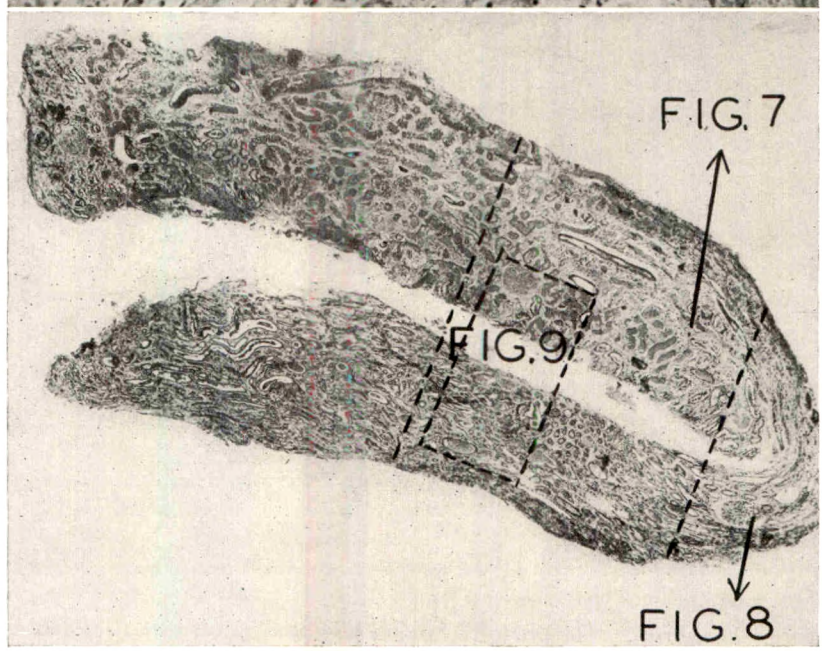


Chart II

The biopsy specimen obtained October 19 measured about 9 mm. in length and consisted of about two-thirds cortex and one-third medulla (Figs. 6, 7, 8, 9). About 4 mm. of the outermost portion of the cortex consisted of necrotic tissue of the same appearance as described



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FIG. 5. Kidney biopsy showing almost completely necrotic, blood and leukocyte infiltrated cortex (acute bilateral cortical necrosis); × 145. (Case III, seventh day after onset of the disease.)

FIG. 6. Kidney biopsy on the eleventh day of the disease. The uppermost segment consists of almost completely necrotic cortex (as in Fig. 5); the intermediate zone is the intact juxtamedullary zone; the lower segment shows retained medulla with casts. (Case III.)

in the previous biopsy. Five or six glomeruli in this portion were almost completely necrotic. Hyaline casts were present in many of the damaged tubules. In the juxtamedullary zone there were two relatively well preserved glomeruli which showed only slightly diffuse hyalinization of the basement membranes. No necrosis or necrobiosis was seen in the medulla which contained considerable amounts of hyaline and many coarsely granulated hemoglobin-stained casts in the loops and the collecting ducts.

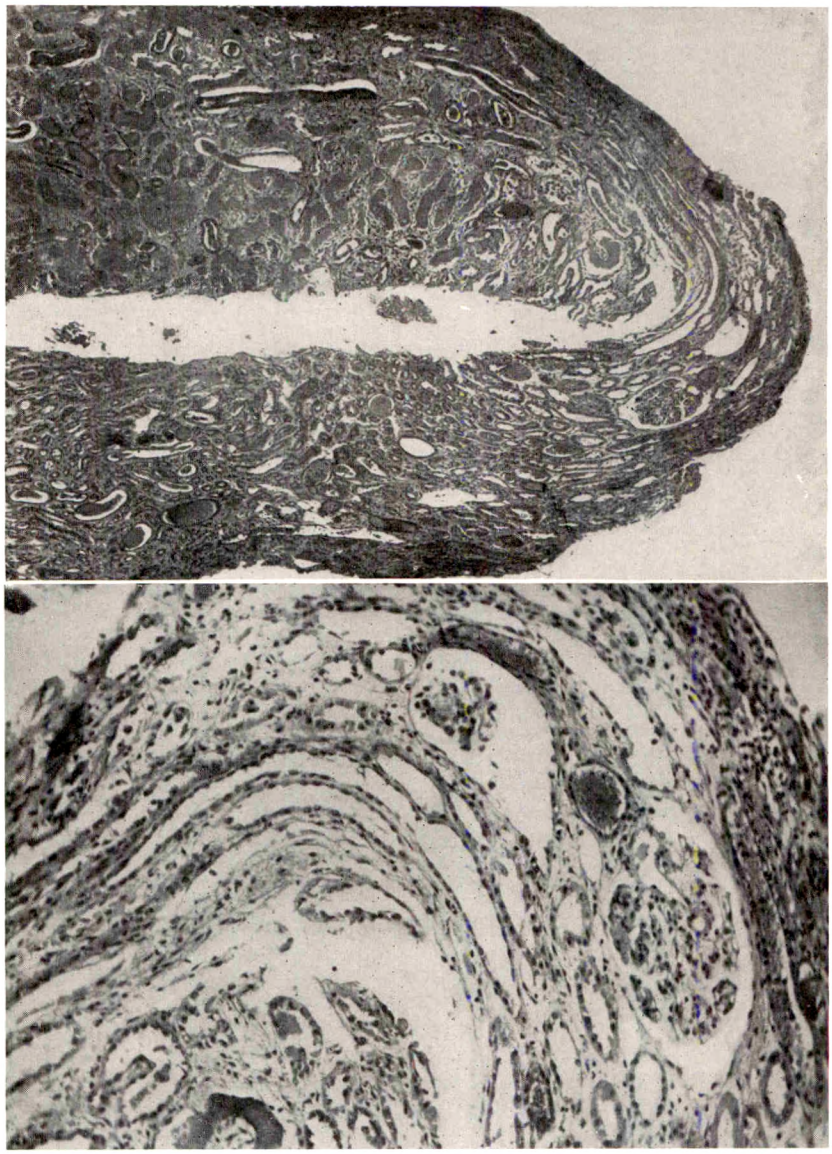


FIG. 7. Same kidney biopsy as that shown in Figure 6. (Case III.)

FIG. 8. Same kidney biopsy as that shown in Figs. 6 and 7; retained juxtamedullary zone with two intact glomeruli and a few hemoglobin-stained casts in distal tubuli; × 145. (Case III.)

In some places these lumina apparently contained pure blood. No inflammatory infiltration was noted in the medulla. No thromboses were found in the arteries in the two biopsies described above. The histologic diagnosis was: almost completely necrotic cortical tissue; medulla with casts of the type seen in tubulointerstitial nephritis.

The two kidney biopsies (kidney biopsy is always performed in the right kidney) and the clinical picture suggested either a large right-sided renal infarct with reflex anuria or large bilateral renal infarcts. In order to solve the question, renal arteriography according to Peirce's method was performed. The picture revealed normal renal arteries with normal, coarse ramifications; the suspicion of embolus or thrombosis in the renal arteries was thus invalidated.

The diagnosis of acute bilateral cortical necrosis would then be the most probable. This diagnosis was established with certainty

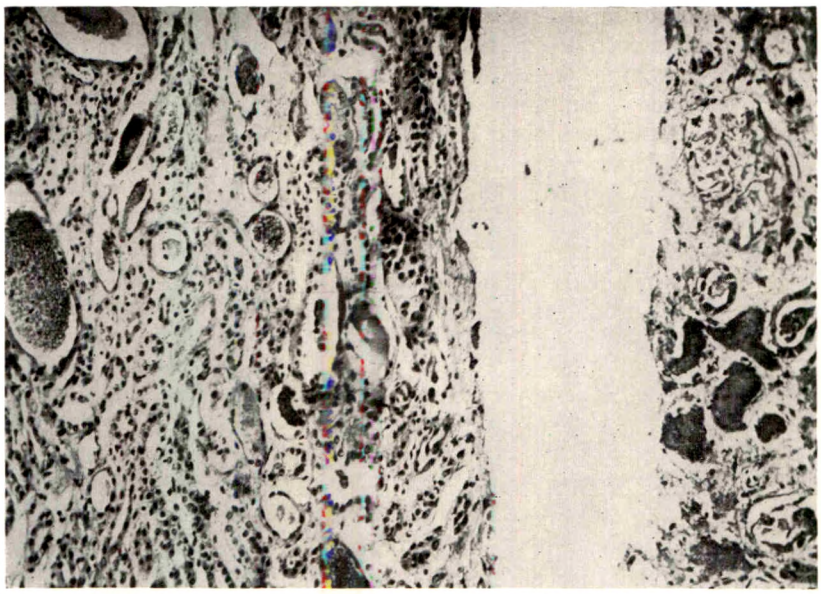


FIG. 9. Same kidney as Figures 6, 7 and 8. Left, retained medulla with hemoglobin-stained casts; right, almost completely necrotic cortex. × 145. (Case III.)

sixty-one days after the onset of the disease when tomograms were taken which showed diffuse calcinosis in the peripheral parts of the cortices of both kidneys.

Treatment and further course: Owing to persistent oliguria with marked impaired renal function (twenty-four hour endogenous creatinine clearance about 0.1 ml./minute) the serum urea level gradually rose to 432 mg. per 100 ml. and the serum creatinine to 24 mg. per 100 ml. As the serum potassium also showed a tendency to rise (Chart II), peritoneal dialysis was instituted on the fourteenth day of the disease (October 22). From the fourteenth to the thirtieth days of the disease the patient was given twelve dialysis treatments; 230 gm. of urea, 10 gm. of creatinine and 5.8 gm. potassium were removed in this manner. During the dialysis period the output of urine began to increase and, at the same time, there was a gradual rise in the twenty-four hour creatinine clearance. On the forty-fifth day it reached a level varying between 10 to 17 ml./minute; when the peritoneal dialysis was discontinued the serum urea had fallen to 250 mg. per 100 ml., the serum creatinine to 9 mg. per 100 ml. Later these values showed a further spontaneous fall to levels of 80 and 4 mg. per 100 ml., respectively.

On the seventy-fourth day of the disease the patient was discharged feeling very well. Three days after her discharge she was readmitted, this time to Surgical Department V, owing to ileus. As this could not be relieved by conservative treatment, surgery was performed and a segment of the small intestine was found to be twisted around a cord-shaped adhesion. Several other adhesions were found between the intestines. The operation was uneventful.

A follow-up nine months after the onset of the disease showed that the twenty-four hour creatinine clearance had risen to about 29 ml./minute; the serum urea was 59 and the serum creatinine was 2.5 mg. per 100 ml.

Summary and comments: Without preceding disease, a twenty-one year old non-pregnant woman suddenly suffered bilateral lumbar pain and complete anuria of five days' duration. On the basis of kidney biopsy the probable diagnosis was acute bilateral cortical necrosis, demonstrated two months after the onset of the disease by x-rays which showed calcinosis in the peripheral parts of the cortex of both kidneys. Kidney function during the first two weeks was practically nil. In the following two weeks peritoneal dialysis was performed and renal function gradually improved. Nine months after the onset of the disease function of the kidney was about one-quarter of normal and the patient was doing rather well.

Bilateral cortical necrosis, a rare disease, was first described by Juhel-Renoy in 1886.[14] It appears most frequently as a complication of pregnancy (bleeding and premature separation of the placenta) and after abortions.[7,26] The dis-

ease, however, may also occur in non-pregnant women and in males and children under conditions (severe hemorrhage, trauma, severe infection, dehydration, diarrhea, vomiting and burns) in which acute tubulo-interstitial nephritis occurs. Duff and Murray[7] have given a good survey of the literature and a complete clinical description. Pathogenesis of the disease is quite obscure but it is probably an extreme variant of acute tubulo-interstitial nephritis. Bilateral cortical necrosis has been produced experimentally in animals by various means: Byrom[4] produced the disease in rats by injecting large quantities of vasopressin; Penner and Bernheim[20] did the same in dogs by means of intraperitoneal injections of adrenaline, and both Navasquez[18] and Trueta et al.[26] produced the disease in rabbits by intravenous injections of staphylococcus toxin.

It is universally agreed that necrosis is of ischemic origin and that it is not due to multiple emboli (Duff and Murray[7]). Trueta et al.[26] and Sheehan and Moore[23] consider that the releasing mechanism must be a prolonged spasm in the interlobular arteries about 0.5 to 1 mm. from their origin from the arciform arteries; thus the juxtamedullary circulation would not be involved by the necrosis. Thromboses often occur in these arteries but they are considered secondary.

Several authors[5,9,17,22,24] have maintained that they have been able to diagnose the disorder clinically, after which the patient recovered. Clinically, the picture can hardly be distinguished from other forms of acute anuria; consequently it is not possible to establish a definite diagnosis without kidney biopsy. As far as we have been able to judge (and according to Trueta et al.) it seems that the diagnosis has hitherto been established with certainty only at autopsy.

With regard to differential diagnosis, acute pyelonephritis, urolithiasis with reflex anuria, acute glomerulonephritis and acute tubulo-interstitial nephritis must be considered. Signs characteristic of bilateral cortical necrosis include bilateral lumbar pain of sudden onset, oliguria-anuria, macroscopic hematuria, fever and absence of dysuria, pollakiuria and pain radiating to the region of the bladder. With regard to the other possibilities, cystoscopy, direct pyelography, renal arteriography and especially kidney biopsy will be decisive.

As the greater part of the glomeruli become necrotic, as in our case, it will undoubtedly prove to be characteristic that renal function is resumed slowly during treatment with dialysis and that it can be restored only to a limited extent. In spite of the small number of glomeruli present in the second kidney biopsy specimen it is interesting to note that the ratio between completely destroyed and preserved glomeruli correspond well to the level of renal function when treatment had been concluded. This was about 15 to 25 per cent of normal. (Chart II.) It would be reasonable to assume that in this respect bilateral cortical necrosis differs from acute tubulo-interstitial nephritis.

Bilateral cortical necrosis is a clinical counterpart to partial corticectomy performed on rabbits by Bing.[2] In Bing's laboratory, Sønder[25] has performed bilateral corticectomies in rabbits after renal hypertension had been established in advance by Goldblatt's method. Sønder showed that when partial corticectomy was carried out in early hypertensive animals with a duration of hypertension up to forty days, it was followed by a prompt and lasting fall in blood pressure. In our case hypertension did not develop during the acute phase of the disease and did not appear within 9 months after the onset of the disease.

SUMMARY

Three cases of acute oliguria-anuria are reported in which kidney biopsy was of great importance in proper interpretation.

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Studies in Serum Proteins*

Agammaglobulinemia in the Adult

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AGAMMAGLOBULINEMIA, the absence of both plasma and extravascular gamma globulin, has recently been found to be associated with unusually poor resistance to infection in certain patients.[1] The defect, which may be congenital or acquired, is more precisely described as a total lack of immunoglobulins since the antibody portion of the beta-2 globulins is absent as well as the gamma globulins, and the defect involves not only acquired antibodies but also such normal inherited antibodies as plasma hemagglutinins. The deficiency appears to be the direct result of an inability to synthesize immunoglobulins since in some such patients administered gamma globulin has been shown to have a normal or prolonged half-life.

Agammaglobulinemia is accompanied by a high mortality and a high incidence of repeated acute and later chronic infections of lung, liver and gastrointestinal tract. It is therefore not surprising that the majority of patients described to date have been children, carefully studied by Janeway and his associates.[2] These cases were believed to represent a congenital form of the disease with sex-linked inheritance, since all affected children were males. However, a rapidly increasing number of reports of agammaglobulinemia in the adult are appearing, representing both the congenital and acquired forms of the syndrome.[3–10] Agammaglobulinemic females are included among these cases. One patient, who will be discussed in the present report, was briefly mentioned some years ago.[11]

The diagnosis of agammaglobulinemia can be easily made because the clinical manifestations are highly suggestive and, once the possibility has been considered, simple laboratory tests quickly establish or refute the diagnosis. The disease provides an excellent example of the value of the pathophysiologic approach since its many clinical and laboratory characteristics may be deduced from a knowledge of the basic defect.

Agammaglobulinemia should be suspected when there is: (1) A history indicating grossly inadequate resistance to infection. Such patients usually give a history of repeated acute episodes of pneumonia, otitis media, meningitis, hepatitis and enterocolitis. Bronchiectasis, chronic hepatitis or sprue-like syndromes may be fully developed when the patient is first seen. (2) Failure to develop clinical immunity or expected laboratory evidence of antibody production after adequate antigenic stimulus. There may be a definite history of two attacks of a disease which normally confers permanent immunity, such as rubella or mumps. Before, during and after an infection with a known specific organism no significant titer of antibody is demonstrable in the serum. Furthermore, no antibody response follows challenge with known potent antigens. Skin tests of the tuberculin type remain negative and the Schick and Dick tests are persistently positive. (3) An unexpectedly normal result with a laboratory test which depends upon abnormalities in serum gamma globulin. The thymol turbidity, cephalin-cholesterol flocculation and colloidal gold flocculation tests are normal although the patient's status suggests they should be abnormal. The Kunkel "gamma globulin" is low when normal or high values would be expected, and the Howe A/G ratio is

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high when normal or low values would be expected. (4) An absence of normally inherited plasma isohemagglutinins.

Diagnosis is established by demonstrating the absence of serum gamma globulin by specific chemical or electrophoretic technics.

METHODS AND SUBJECTS

Clinical studies in Cases III and IV were carried out at the Michael Reese Hospital, in the former instance with the cooperation of Dr. Boris B. Rubenstein, and in the latter with the cooperation of Dr. Norman F. Boas and the late Dr. Henry S. Guterman.

Serum protein patterns were obtained by the chemical micromethod of Wolfson and Cohn,[12] by filter paper electrophoresis employing apparatus and technics developed in this laboratory,[13] and by free electrophoresis in the Aminco-Stern (Case IV) or Perkin-Elmer (Case II) instrument.* Free electrophoresis was conducted in barbital buffer, pH 8.6, ionic strength 0.1. For paper electrophoresis the buffer had the same pH but the ionic strength was 0.05, and 10 per cent of ethanol (v/v) was added to inhibit mold and adsorption. Electropapergrams were dried in an oven at 100°C., fixed in bichloride of mercury and simultaneously stained with bromphenol blue. Excess bromphenol blue was then removed by exhaustive washing in 0.5 per cent aqueous acetic acid, the paper dried in an oven and the indicator dye converted to the blue form by exposure to ammonia. The electropapergram was then scanned either with a Gamma I scanning photometer or with the Wolfson-Skinner strip-scanning adapter for the Beckman DU spectrophotometer. The resulting curves were measured planimetrically in the usual manner. Other chemical determinations employed routine current laboratory technics.

CASE REPORTS

The typical history of an agammaglobulinemic patient is a stormy one and is well illustrated by Case I, which will therefore be presented in some detail.

CASE I. The history prior to study in this Unit is best summarized chronologically as follows: Age one to three years, apparently normal; four years, bilateral purulent otitis media, many exacerbations in subsequent years; five years, scarlet fever; seven years, varicella, rubella, pertussis and acute mastoiditis; ten years, suppurative cervical adenitis, poliomyelitis; eleven years, suppurative cervical adenitis, infectious hepatitis, lobar pneumonia; twelve years, rubella, lobar pneumonia; thirteen years, lobar pneumonia, suppurative cervical and inguinal adenitis, diagnosis of bronchiectasis established; fourteen to seventeen years, many episodes of pneumonia; seventeen years, operation for chronic sinusitis; eighteen years, two attacks of lobar pneumonia, lobectomy for severe symptomatic bronchectasis; nineteen years, four attacks of lobar pneumonia, purulent meningitis, diagnosis of hepatic cirrhosis established; twenty years, two attacks of lobar pneumonia. Throughout these twenty years he also had a multitude of minor infections and ill defined febrile episodes. The diagnosis of agammaglobulinemia was made in September, 1953, when the patient entered the City of Detroit Receiving Hospital because of lobar pneumonia.

TABLE II

SERUM PROTEINS IN AGAMMAGLOBULINEMIA, ILLUSTRATING THE CHARACTERISTICALLY HIGH HOWE A/G RATIOS

	True A/G Ratio	Howe A/G Ratio
Agammaglobulinemia		
Case I	3.3	13.4
Case II	1.6	3.5
Case III	3.1	10.3
Case IV	1.3	4.8
Normal adult		
Average value	1.2	2.3

The patient's parents, sister and four brothers were living and well. One brother had died at the age of one, apparently of pneumonia. Physical examination disclosed a chronically ill and somewhat underdeveloped white male. He was 67 inches tall and weighed 118 pounds. He had chronic bilateral otitis media, extensive bronchiectasis of the right lower lobe, hepatosplenomegaly and generalized shotty lymphadenopathy.

Table I summarizes the pertinent laboratory findings. Needle biopsy of the liver disclosed early portal cirrhosis without lipidosis. A lymph

* For the free electrophoresis analyses we are indebted, respectively, to Dr. Ivan F. Duff of University Hospital, Ann Arbor, Mich., and to Miss Miriam Reiner of Mount Sinai Hospital, New York, N. Y.

node biopsy revealed normal node architecture with mild reactive reticulum cell hyperplasia. A bone marrow study showed no significant abnormalities. Chemical fractionation of the serum proteins and paper electrophoretic studies disclosed a total absence of gamma globulin. (Fig. 1.)

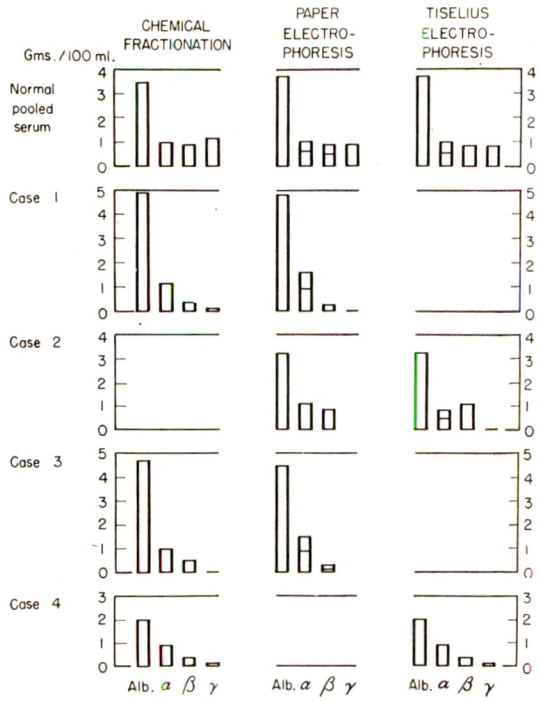


FIG. 1. Agammaglobulinemia; serum protein fractionation studies.

CASES II, III AND IV. These three adult males with proved agammaglobulinemia ranged in age from twenty-six to thirty-three years. All had had frequent infections since early childhood, with bronchopulmonary involvement predominating. One had definite bronchiectasis. Attempts to detect an antibody response to administered antigens were uniformly unsuccessful. Howe A/G ratios were all unusually high. Chemical fractionation of the serum proteins by the method of Wolfson and Cohn, by classical Tiselius electrophoresis, or by paper electrophoresis disclosed an absence of circulating gamma globulin. (Table II, Fig. 1.)

RESULTS

Protein Fractionation Studies in Patients with Agammaglobulinemia. Figure 1 shows the complete absence of gamma globulin from the sera of all these patients. The beta-2 globulin concentration, although somewhat difficult to estimate by paper electrophoresis, clearly was low in Cases I and III. Other fractions show only those abnormalities anticipated from the patients' clinical status. High serum alpha globulin would be expected because of the presence of chronic suppuration in Cases I, III and IV and because of recent acute infection in Case II. Hypoalbuminemia in Case IV was found shortly before the patient died of chronic suppurative pulmonary disease. Apparently, the primary changes in serum proteins involve only the beta-2 and gamma globulin fractions, a finding consistent

TABLE I

PERTINENT LABORATORY FINDINGS IN CASE I

Serum total protein	6.55 gm. %	Kunkel gamma globulin test	1 unit
Howe serum albumin	6.10 gm. %	45-minute bromsulfalein retention	20 %
Howe serum globulin	0.45 gm. %	Serum cholesterol	250 mg. %
Howe "A/G ratio"	13.4	Blood hemoglobin	14 gm. %
Serum bilirubin	0.4 mg. %	Blood urea nitrogen	14 mg. %
Urine bilirubin	Negative	Congo Red test	Negative
Urine urobilinogen	Negative	Blood type	A_1
Schick test	Positive	Anti-b and anti-a_2 plasma isohemagglutinins	Absent
Tuberculin test	Negative		
Cephalin flocculation test	Negative		

	Total Leukocytes	Granulocytes	Lymphocytes + Monocytes
Normal range	5,000–10,000	3,000–5,800	1,785–3,500
Patient, afebrile	9,100	4,368	4,550
Patient, febrile	19,800	16,434	3,168

with their postulated special function and their site of synthesis.

Howe Fractionation Values in Agammaglobulinemic Patients. When sodium sulfate is added to serum in an amount sufficient to give a final concentration of 21 per cent, a portion of the serum proteins precipitates. In this original technic of Howe the protein precipitated is designated "globulin," while that still in solution is termed "albumin." However, the "albumin" of the Howe method actually includes both the true albumin and part of the alpha globulin as estimated by the Wolfson-Cohn chemical fractionation procedure or by free or paper electrophoresis. The "globulin" of the Howe method includes largely the beta globulin and the gamma globulin of modern methods.

From these considerations it is apparent that the "albumin"/"globulin" (A/G) ratio of the Howe method approximates

$$\frac{\text{albumin plus alpha globulin}}{\text{beta globulin plus gamma globulin}}$$

as estimated by the Wolfson-Cohn chemical fractionation or by electrophoresis. In agammaglobulinemia the denominator is small because of the absence of the gamma globulin component and the reduced beta-2 globulin devoid of immunoproteins. In relatively healthy patients with this disorder the numerator will have its normal value; but even if albumin is reduced, as in Case IV, the rise in alpha globulin concentration which is usually associated with a fall in albumin will tend to minimize any decrease in the numerator. The patient with agammaglobulinemia therefore has a high Howe "albumin"/"globulin" ratio because the numerator is relatively normal while the denominator is greatly reduced. In three of the cases herein reported the routine laboratories processing the Howe fractionation values suspected laboratory error because the A/G ratios were so high.

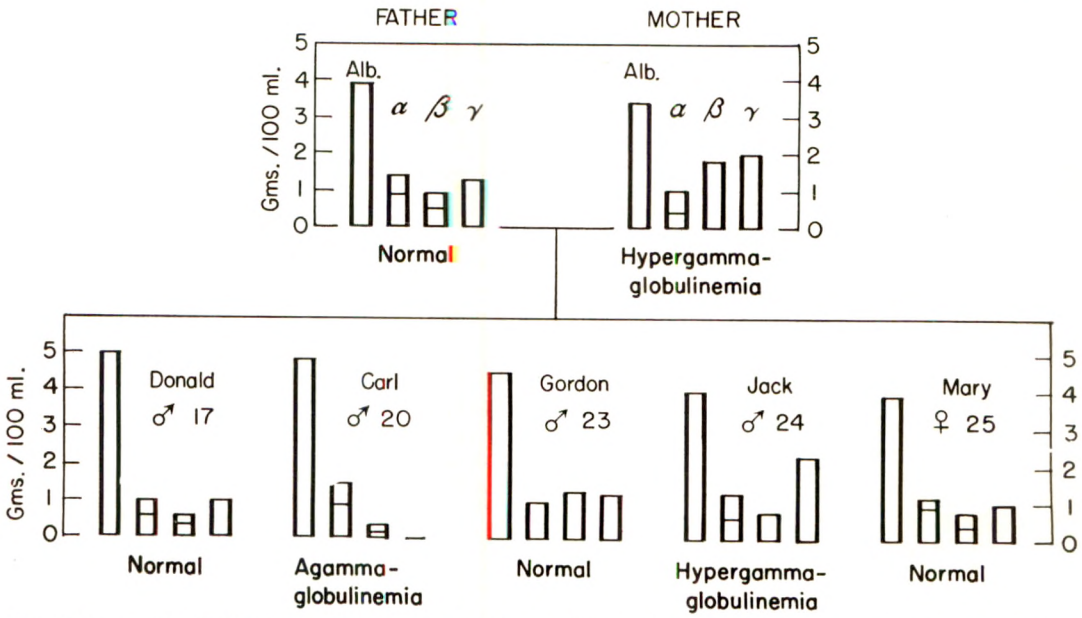


FIG. 2. Serum protein fractionation studies on the family of the patient in Case I, demonstrating hypergammaglobulinemia in two members.

Protein Fractionation Studies in Unaffected Relatives of Patients with Agammaglobulinemia. Serum proteins were examined in unaffected relatives of two of our patients (Cases I and III), and in a number of the patients studied by Janeway and associates.[14] Abnormal patterns have been found only in the family of our Case I. His mother and one of his brothers both showed marked hypergammaglobulinemia and hyperbeta-2-globulinemia, which could not be explained clinically. (Fig. 2.)

Other Findings in Agammaglobulinemic Patients Which Suggest Absent Circulating Gamma Globulin or Antibody. Naturally occurring plasma isohemagglutinins were absent in Case I and in patients studied by Bruton and Janeway. Since

these substances are known to be beta-2 globulins, their absence correlates well with the electrophoretic finding of a low beta-2 globulin value. All reported patients have been blood types A or O but, because of absent isohemagglutinins, all are presumably universal recipients.

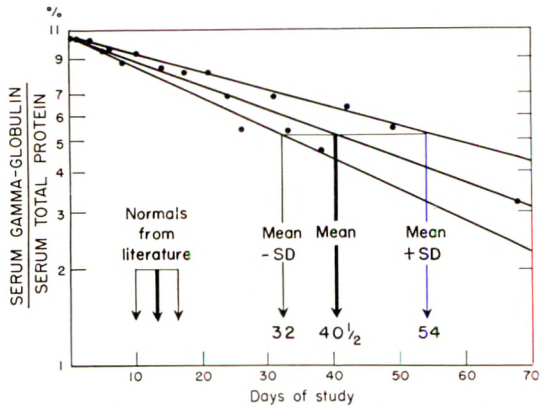


FIG. 3. Case I. Half-life of administered human gamma globulin; normal values are based on the half-life of I-131 labeled gamma globulin. Recent evidence indicates that such values are probably low. Therefore, the half-life of the unlabeled gamma globulin administered to this patient is probably not abnormally prolonged.

Another anticipated observation is the failure of intercurrent disease, particularly liver disease, to produce abnormal protein flocculation tests, because all appear to require some elevation or abnormality of gamma globulin before they become positive. This is well illustrated in Case I. (Table I.) Patients with agammaglobulinemia do have erythrocyte sedimentation rates consistent with their state of health or illness because fibrinogen, the chief plasma protein determinant of the sedimentation rate, shows its usual increase in response to illness or injury.

Defective Response to Antigenic Challenge. Agammaglobulinemic patients are incapable of responding to antigen by producing specific antibodies, whether the antigenic stimulus is a naturally occurring disease or a purified antigen. The common skin tests do not change after appropriate stimulation with antigens. Three patients of this series (Cases I, II and III) produced no circulating antibody after repeated administration of typhoid vaccine. The Schick tests were positive. The tuberculin test was negative in Case I. In the cases reported by Bruton and Janeway there was no antibody response to administered pneumococcal polysaccharide or typhoid vaccine. In one case no complement-fixing antibodies for mumps could be demonstrated in the serum twenty-two days after a typical attack. Schick tests were noted to remain positive in spite of repeated administration of diphtheria toxoid. This phenomenon is responsible for a characteristic clinical feature of the syndrome, namely, the occurrence of more than one attack of a disease which normally confers lasting immunity, such as mumps, pertussis and measles.

The response to vaccination is unanticipated and forms almost the only finding in agammaglobulinemic patients which is at all inconsistent with expectation. Janeway et al.[2] have indicated that a primary "take" may be obtained and that if such a patient is revaccinated an accelerated reaction indicative of partial immunity may be seen. This, of course may represent a type of adaptation not involving immunoglobulin formation and, if so, suggests the presence of previously unrecognized factors in resistance to infectious disease.

Blood Lymphocyte Counts and Lymph Node Histology. Experimental studies have suggested that fixed tissue lymphocytes either may become liberated as blood lymphocytes or, while still located in the lymphoid tissue, may undergo cytolysis and give rise to blood antibodies by shedding their surface protein film. It has been suggested that the relative rates of these two processes is dependent upon adrenal glycocorticoids, higher levels favoring the cytolytic process.[15]

In Cases II, III and IV peripheral blood lymphopenia was persistently present, the absolute lymphocyte counts ranging from 798 to 1,117 per cu. mm. The absence of associated eosinopenia or neutrophilic leukocytosis clearly showed that an increased level of adrenal corticoids was not the cause of this lymphopenia. Its probable origin was indicated by the changes found in the lymph nodes of two of these patients. In both, the nodes appeared markedly hypoplastic and severely deficient in tissue lymphocytes (lymphoblasts). By contrast, the remaining apparently intact reticular framework was unusually prominent. Janeway and Bruton have examined nodes, selected from a number of regions, and have found either a similar deficiency of tissue lymphocytes or an absence of germinal centers.[16] Either finding suggests diminished lymphopoiesis, since the prominence of the germinal center is believed to be an index of the rate of lymphocyte formation. Thus in these patients it

would appear logical to explain the absence of gamma globulin, the blood lymphopenia and the quantitative lack of tissue lymphocytes seen at biopsy or implied by the absence of germinal centers as due to greatly reduced lymphopoietic activity. Because this view fails to explain why there should be a relative deficiency of blood lymphocytes and a complete absence of blood immunoglobulins, it would seem that defective release of antibody from templates must also be postulated.

In Case I, however, the peripheral blood lymphocyte count was in the high normal range when the patient was afebrile, and during infections it changed in the normal manner. Several lymph nodes and diffuse lymphoid tissue from the Waldeyer ring area of the nasopharynx were found to be normal except for some sinusoid dilatation and reticulum cell hyperplasia in these dilated sinusoids.

The agammaglobulinemia of Case I was quite as complete as that of the other patients in spite of normal-appearing lymphoid tissue. Since no evidence of slowed lymphopoiesis could be found, this patient in contrast to the others described, may have some qualitative disturbance in the protein-synthesizing mechanism of his tissue lymphocytes. This interpretation is supported by the isolated hypergammaglobulinemia observed in persons whose relationship makes it plausible to assume that they are heterozygous carriers of a recessive gene which produces agammaglobulinemia without lymphopenia in a homozygote.

Absence of Effect of ACTH and Other Medications on Serum Proteins and Peripheral Blood Counts. ACTH was administered to one patient with the lymphopenic type (Case II) and one patient with the non-lymphopenic type (Case I) of agammaglobulinemia. One patient with each of the types of agammaglobulinemia responded to test doses of ACTH with normally intense eosinopenia and lymphopenia. Following intensive courses of treatment with ACTH gel for seven to eight days, no detectable amounts of gamma globulin were noted. Neither relative corticoid deficiency nor excess seems to provoke or relieve agammaglobulinemia of either type.

Because hypogammaglobulinemia has occurred in experimentally induced pyridoxine deficiency in animals, large doses of pyridoxine (100 mg./day), with and without whole vitamin B complex, were administered for prolonged periods to two patients (Cases I and II) without effect. Large amounts of desoxyribonucleic acid were fed to one patient because of the work of Dustin[17] who found that it caused thymic and lymphatic hyperplasia. No significant change was noted.

Rate of Disappearance of Administered Human Gamma Globulin in Agammaglobulinemia. The half-life of administered gamma globulin in several children with agammaglobulinemia was reported to range from twenty to thirty days,[14] values slightly longer than normally found in children. Figure 3 demonstrates the apparently prolonged half-life of homologous gamma globulin in Case I. In Case III the rate of disappearance of administered gamma globulin was found to be within the normal range for adults. These observations exclude rapid catabolism of gamma globulin as an explanation for this syndrome. They also suggest that as plasma-globulin levels fall the relative amount utilized per unit time decreases.

DISCUSSION

Physiologic Basis of Therapy in Agammaglobulinemia. Normally about 13 per cent of the total serum protein consists of gamma globulins, chiefly immunoproteins, together with a considerable quantity of "non-specific" gamma globulin of as yet unknown significance. Certain antibodies, including blood group isohemagglutinins, typhoid "O" agglutinins and true Wassermann reagin are also found in the beta-2 globulin fraction.[18] The normal adult has approximately 25 gm. of circulating gamma globulin in dynamic equilibrium with an equal quantity of extravascular gamma globulin.[19] The serum level of gamma globulin appears to be closely related to adaptive processes. It is relatively high at birth, presumably due to transplacental transmission of antibodies, falls for several months, and thereafter rises slowly as the individual comes into contact with immunizing diseases. Adult levels are not reached for over two years.

The half-life of gamma globulin, as determined by the administration of homologous I-131 labeled gamma globulin,* is 13.1 ± 2.8 days in adults, and somewhat longer in children, 20.3 ± 4.1 days.[20] The normal adult, therefore, apparently catabolizes about one-thirteenth of

* The validity of values obtained by this method has recently been questioned. Armstrong et al. have reported turnover times of nineteen to twenty-three days using S^{35}-labeled yeast (*J. Lab. & Clin. Med.*, 44: 762, 1954).

his 50 gm. of gamma globulin, approximately 3.8 gm., each day.

Replacement therapy with human gamma globulin has been reported effective in controlling the principal manifestations of this syndrome. Affected children have been maintained free of sepsis on weekly injections of gamma globulin and prophylactic penicillin.[1,14] It is apparently neither necessary nor practical to achieve normal blood levels of gamma globulin to prevent infection. Gitlin has noted that levels of only 100 to 150 mg. per 100 ml. are effective.[14] This is fortunate since it may be calculated that an adult would require about 25 cc. of the commercial 15 per cent gamma globulin solution per day for complete replacement. The material cannot be administered intravenously because of severe hypotensive reactions, and intramuscular injections of over 20 ml. are prone to cause chills, fever, myalgia and faintness. Since the virus of homologous serum jaundice is not associated with the gamma globulin fraction, there is little danger of serum hepatitis, even with prolonged administration.

Site of Origin of Immunoglobulins. The existence of a disorder in which immunoglobulins are absent and other serum proteins are unaffected indicates a special site and mechanism for synthesis of immunoglobulin. The lymph nodes and spleen are known to be important sources of antibodies but the specific cell type which forms antibodies has not been established with certainty. Despite much investigation, various workers favor reticuloendothelial cells, immature tissue lymphocytes, plasma cells or several of these as the synthesizing organ.[21,22] Simultaneous absence of immunoglobulins and evidence of slowed lymphopoiesis in one group of patients favors the immature tissue lymphocyte; however, the existence of a non-lymphopenic type of agammaglobulinemia shows that all factors are not yet known.

Differential Diagnosis of Hypogammaglobulinemia. A subnormal level of plasma gamma globulin results from a disproportion between its rate of synthesis and the rate at which it is utilized. Once the rate of synthesis becomes less than the rate of utilization, the level would fall to zero unless the decrease in concentration also brought about a diminished rate of utilization. The prolonged half-life of injected gamma globulin in agammaglobulinemic subjects suggests that utilization is slower at low levels.

A subnormal rate of gamma globulin synthesis may occur when normal lymphoid tissue receives an insufficient supply of precursors or when a normal precursor supply is delivered to hypofunctioning lymphoid tissue. Knowledge of the precursors of gamma globulin is relatively incomplete. Within wide limits, synthesis is not critically dependent upon protein intake in normal subjects. In certain rare patients believed to have a selective type of hepatic dysfunction,[23] hypogammaglobulinemia even at high levels of protein intake has been described. Apparently, the liver makes some unknown metabolite essential for subsequent formation of gamma globulin by lymphoid tissue. This essential metabolite would appear always to be in limited supply, since hypogammaglobulinemia may occur when an abnormal protein is produced at a rapid rate (v. seq.), even though the lymphoid tissue is histologically normal. In suitably designed animal experiments, hypogammaglobulinemia may be induced by deficiency of pantothenic acid or pyridoxine or by the pyridoxine antagonist, desoxypyridoxine.[24] The required degree of deficiency is severe and clinical hypogammaglobulinemia has never been shown to result from a deficiency in either of these two vitamins, which are essential in coenzymes of protein synthetic processes.

Hypogammaglobulinemia which results from a deficient supply of precursors is never complete; and since minimal levels of functionally active immunoglobulins apparently suffice for normal resistance, unusual susceptibility to infection is not prominent in the disorders just cited. The major symptoms are usually edema and ascites, the result of an associated diminution in the synthesis of plasma albumin. The available but still scanty data are consistent with the expected normal or prolonged half-life of injected gamma globulin or albumin.

Hypogammaglobulinemia is not uncommonly seen in multiple myeloma with a beta globulin or M-component abnormality, and may well be due to diversion of gamma globulin precursors for the synthesis of the abnormal component. The electrophoretic pattern of an unusual case of apparent agammaglobulinemia associated with an M-component myeloma is presented in Figure 4. The patient had a history of many bouts of sepsis, particularly pneumonia, since the onset of the disease. He failed to develop antibodies in response to typhoid vaccination, and the tuberculin and histoplasmin skin tests were negative. However,

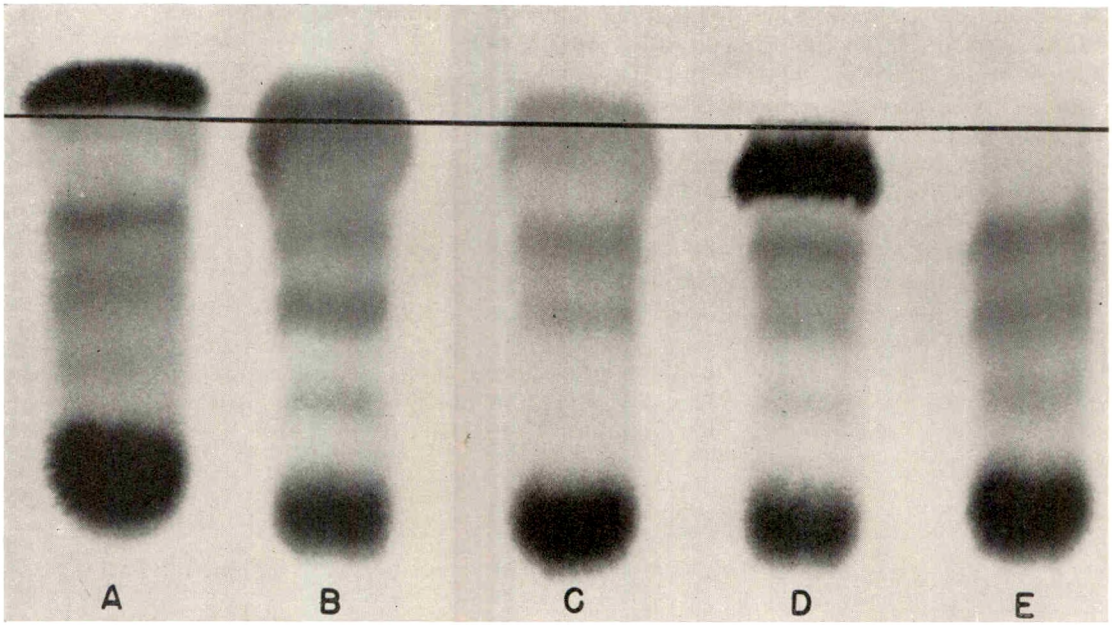


FIG. 4. Case I. Electropapergrams of 5 sera, illustrating gamma globulin abnormalities. A, gamma myeloma, the abnormal gamma globulin fraction consisting almost entirely of cryoglobulin; B, hypergammaglobulinemia in a case of disseminated lupus erythematosus; C, normal serum; D, M-component myeloma with agammaglobulinemia, the abnormal globulin consisting largely of pyroglobulin; E, congenital agammaglobulinemia. Transverse line indicates point of application of serum.

isohemagglutinins were present in the serum, indicating that the immunoglobulin-synthesizing defect was not inherited or complete.

Given an adequate supply of essential precursors, deficient production of gamma globulin may still occur if the lymphoid tissues function inadequately. The total mass of the synthesizing tissue may be greatly reduced by cancerous replacement, by radiation damage or by certain radiomimetic chemicals, notably benzol and nitrogen mustards. A quantitative defect in lymphoid structures may also be congenital, as is probably true of Cases II, III and IV in this report. In addition, the data in this paper suggest that more subtle qualitative hypofunction of lymphoid structures may occur (Case I).

Hypofunction of the lymphoid structures concerned in gamma globulin synthesis is not ordinarily associated with a similar disturbance in albumin formation, so that ascites and edema are not an integral part of the symptomatology. It may, however, lead to complete agammaglobulinemia with its characteristic clinical picture. In these disorders, the half-life of injected gamma globulin is normal or prolonged and, if agammaglobulinemia is present, complete failure of synthesis is shown by the absence of an antibody response to antigenic challenge.

Abnormally rapid loss of gamma globulin from the plasma is the most common cause of clinical hypogammaglobulinemia. In the nephrotic syndrome there is excessively rapid transcapillary loss of gamma globulin, with a parallel loss of albumin, in the urine. Hypogammaglobulinemia as well as hypoalbuminemia has also been described in rare cases of familial idiopathic dysproteinemia,[25] an interesting disorder resembling the nephrotic syndrome but without renal abnormalities.

Other theoretic causes for an increased rate of gamma globulin removal include fixation in antigen-antibody reactions and an increased rate of protein catabolism. The fall in plasma gamma globulin often seen in Cushing's syndrome and reproducible in normal adults with corticotropin may result from an increased rate of protein catabolism, overshadowing a less marked increase in the rate of gamma globulin synthesis.

SUMMARY

Agammaglobulinemia is a syndrome which may be due to congenital or acquired factors. The clinical manifestations are readily explained by failure of immunoglobulin synthesis due to a defect in the synthesizing tissues. Such patients have a history of grossly inadequate resistance

to infectious disease, and fail to produce antibodies in response to natural or artificial antigenic challenge.

Four cases of adult agammaglobulinemia are described. All patients were men. In three cases agammaglobulinemia was associated with a quantitative deficiency in tissue and peripheral blood lymphocytes, and relatives were apparently unaffected. One patient had no apparent lymphocyte abnormalities, and relatives of both sexes manifested hypergammaglobulinemia.

The disorder is readily diagnosed by serum protein electrophoresis or chemical fractionation studies and is believed to be amenable to specific replacement therapy with human gamma globulin.

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Review

Studies of Ulcerative Colitis*

III. The Nature of the Psychologic Processes

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In previous papers I have discussed the various psychosomatic hypotheses concerning ulcerative colitis in terms of their consistency with data available as to the nature of the somatic processes.[19,20] It was concluded that the anatomic and functional locus of the disease is the lining surfaces (mucosa and submucosa) of the colon and ileum, and that the nature of the process more closely resembles a reaction to a microscopic or molecular-sized noxious agent than a bolus-sized agent. Evidence was presented to show that the primary reaction is at this level of tissue organization, with hyperemia, edema, exudation and secretion as prominent components, while the behavior of the colon as a whole, as reflected in the character of the bowel movements, diarrhea or constipation, is dependent upon the location and severity of the local reaction. When the surface reaction is restricted to rectum or rectosigmoid, constipation is the rule, whereas when the process involves proximal bowel or extensive segments of bowel, diarrhea is usual. Such relationships are most readily detected at the inception of the disease and, once bowel has been damaged, other factors influence symptomatology in unpredictable ways. In light of these facts it was concluded that none of the psychosomatic processes so far advanced properly identify the somatic processes and therefore require re-evaluation.

In this paper I propose to examine the available data concerning psychologic processes in ulcerative colitis, summarizing the literature as well as my own observations. In doing so I am influenced by the fact that few physicians have had or ever will have the opportunity to obtain this kind of information in more than a few patients and therefore there is a need to bring together in one place all the facts as well as some indication of the number and variety of patients upon which such facts are based. The frame of reference employed is a broad one. In studying psychologic processes attention is directed to one aspect of the total functioning of the human being, that which can be examined by psychologic means and expressed in psychologic terms. We deal with the observable phenomena of intrapsychic and interpersonal processes, including ontogenetic aspects. This material is then analyzed for any consistent patterns in this group of patients as a whole, as well as in relation to exacerbations and remissions of the pathologic processes in the colon. No assumption is made that such correlations have etiologic significance. We are at this time attempting only to establish the existence and nature of conditions which may be contributory or even necessary yet may not be sufficient in themselves for the development of ulcerative colitis. We do not concern ourselves here with the problem of psychogenesis in the sense of psychologic processes *causing* ulcerative colitis. This kind of a study may be compared to the epidemiologic approach to malaria, in which information about geography, climate, breeding conditions of mosquitoes, the life cycle of the mosquito, the conditions necessary for survival of the malarial parasite, the conditions necessary for the transmission of the parasite from vector to human host and back again, the factors of host resistance, and many others all combine to define the conditions necessary and sufficient for the development of the disease malaria. The identification and elucidation of any one condition represents an important advance in the understanding of the disease

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but does not "explain" pathogenesis or establish etiology. Similarly, psychologic study of patients with ulcerative colitis represents but one step in the clarification of conditions necessary, but not necessarily sufficient, for the development of the disease. Whether or not such an approach will prove fruitful in the study of any particular disease entity cannot be established until the study has been carried out.[23,24]

The matter of significance must also be discussed here. The fact that certain phenomena are observed consistently, or even commonly, in a group of patients justifies their further study. Critics of the psychologic approach often dismiss as of no significance the observation that some psychologic event commonly precedes the development of an organic process by pointing out that many people experience the same kind of psychologic event without developing the disease, and therefore the two cannot be related. This, however, would be the same as arguing that the typhoid bacillus cannot be a significant factor in typhoid fever because many people who are exposed to the organism do not develop the disease. It ignores the vulnerability of the individual. Pettenkoffer's dramatic attempt to refute the significance of vibrio cholera by publically ingesting a culture of living organisms is a case in point.

And finally it is necessary to stress the importance of method. It is not possible to evaluate the importance of psychologic factors unless the material is examined by methods appropriate for that kind of evaluation.

MATERIAL AND METHODS

This paper is based upon examination of forty-four published reports of psychologic data in more than 700 patients with ulcerative colitis, plus observations in thirty-nine patients of my own, not all of whom were studied with the same thoroughness. Many of these reports represent pooled impressions of a large number of patients,[48a,55,56] while others represent single case reports. Of this large published material there are only forty-one reasonably detailed protocols which permit the reviewer opportunity for examination of more primary data. Some papers do not mention the number of cases upon which the conclusions are based. And in many it is very difficult to know which interpretations are derived from the writer's own observations and which are reflections of earlier writings.

No attempt will be made to review or summarize each paper. Instead I have selected for critical evaluation certain categories of psychologic processes and have examined the literature as well as my own data for material bearing on the phenomena under consideration. In so doing it was sometimes necessary to use and interpret data from the publications of other writers in ways which they did not emphasize.

ANAMNESTIC AND PSYCHOANALYTIC STUDIES

Anamneses vary from relatively superficial reports by observers with little psychologic training to histories obtained by psychoanalytically trained physicians. While quite a few patients with ulcerative colitis have been analyzed and considerable material is available from such sources, reasonably detailed protocols of psychoanalyses are still few.[13,14,25,38,51,52,66] The psychoanalytic method, however, involving as it does long and detailed study of the patient, clearly establishes that the major psychodynamic trends long antedate the development of clinically manifest ulcerative colitis. The often repeated assertion that the psychologic characteristics of these patients are the consequence of the severe and debilitating disease finds no support when patients are studied for years, including long periods of complete remission.

Personality Structure

Under this heading are grouped the more or less characteristic behavioral patterns of an individual, the person's peculiar mode of dealing with psychic tension.[3]

A high proportion of patients are described as manifesting obsessive-compulsive character traits, including neatness, orderliness, punctuality, conscientiousness, indecision, obstinacy and conformity. Along with these are often noted a guarding of affectivity, overintellectualization, rigid attitudes toward morality and standards of behavior, meticulousness of speech, avoidance of "dirty" language, defective sense of humor, obsessive worrying and timidity. Alexander[1] in particular has stressed the prominence of the so-called anal characteristics. (See references 2, 5–8, 10, 11, 13, 17, 25, 31, 32, 34, 38, 43, 47, 48*a*, 50, 51, 55–59, 64, 66, 68–71, 78, 80.) The deviations from this pattern are of interest. A few patients are conspicuously sloppy, disorganized, dirty and unkempt—some of these are psychotic.[25,41,42,80] During the active disease and during analysis the patient may betray an extraordinary preoccupation with feces which

contrasts with his usual fastidiousness.[25,45,46,64,66] Some patients are petulant, querulous, demanding and provocative, but well directed aggressive action and clear-cut expressions of anger are uncommon. (See references 7, 25, 31, 34, 35, 51, 55, 57–59, 69, 80.) Many writers have been impressed with the extreme sensitivity of these patients, their almost uncanny perception of hostility or rejecting attitudes in others. They are easily hurt, constantly alert to the attitudes and behavior of others toward them, tend to brood and withdraw. Much activity is devoted to warding off or avoiding rebuffs, including placating attitudes, submission, politeness, attempt to please and conform, seductive behavior. Others use denial, remaining proud, nonchalant, haughty and aloof. (See references 2, 5, 7, 13–17, 25, 31, 34, 35, 38, 43, 47, 48*a*, 55, 57, 58, 64, 68–71, 78, 80.)

All observers have in various ways described these people as "immature." This has generally referred to the prominence of dependent attitudes, to their restricted capacity to tolerate frustration, to their relatively low capacity to assume responsibility in family or work, to their sexual immaturity, to the restricted character of their relationships with people. One is less likely to find among patients with ulcerative colitis a person who was highly successful before the illness began. Some patients give an outward appearance of energy, ambition and efficiency but this usually proves to be a thin veneer which hides unreasonable feelings of inferiority, an acute sense of obligation, a need to achieve some sense of security.[80] They avoid chances or dealing daringly with their environment. Such people are often admired for their virtue, morality and high standards.

Although descriptions and case protocols in the literature leave the impression that this characterization of the ulcerative colitis patient is accurate, there are few figures of actual incidence. I found obsessive-compulsive character traits to be prominent in twenty-six of twenty-seven patients. None, however, had a well developed obsessive-compulsive neurosis, and all showed some to considerable decrease in some of the obsessive-compulsive traits during the active illness. Twenty-three of twenty-five were ingratiating, placating, submissive, attempted to make an acceptable appearance, were very sensitive, avoided direct demands and had low self-esteem. The other two patients differed from this description mainly in being more petulant, querulous and overtly demanding. Wittkower[80] classified forty patients into three groups. Twenty-three patients were rigid compulsive characters with a strong sense of inferiority. A second group of twelve, all women, differed chiefly in a more overt display of stormy emotions, were more noisy, petulant, argumentative and exhibitionistic but in other respects resembled the first group. Five patients fit in no consistent group but showed much social anxiety, were shy, depressed and terrified of transgression.

Comment: In recent years attempts to establish typical personality profiles have fallen into disrepute, since usually only the more manifest character traits are considered and dynamic factors are not recognized. Yet this attitude may go too far in underestimating the value of this type of data as a source of information indicating certain dynamic trends. With better understanding of the factors involved in character formation one not only may find clues to common dynamics but also to factors which may be covariant to both the psychic and somatic processes. It would be misleading to say that the character traits here listed are specifically associated with ulcerative colitis. Most people with such character traits do not have ulcerative colitis. But given the diagnosis of ulcerative colitis, the possibility of describing the major character traits of the patient without even seeing him is excellent.

Relationship with People

Patients with ulcerative colitis also reveal a rather consistent pattern of relationship with people. On the one hand they appear to have a quite "dependent" relationship on one or two persons, on the other hand they seem to lack the capacity to establish warm, genuine friendships with others.

In the literature this has been described in such terms as "dependence," "overdependence," "infantile dependence," "abnormal attachment," "ambivalent dependence," "parent controlled," "fixation on parent," "symbiotic relation." (See references 1, 2, 5–7, 9, 13–17, 25, 31, 34, 35, 37, 38, 41, 43, 48*a*, 50, 51, 55, 57–59, 64, 66, 68–71, 78, 80.) In most instances this intense relationship is with a parent or a parent substitute. Many of the writers point out that, if and when these patients marry, the spouse often fulfills such a role. Lindemann[42] has placed particular emphasis on the key role of some per-

son in the psychosocial orbit of the patient. He describes this person as one whose presence in the orbit of human relationships is indispensable for the patient. Sperling[67] (speaking of "psychosomatic" cases in general and not ulcerative colitis alone) described the patient as living "in an emotional symbiosis with one object in the environment, who does not have to be the actual mother but who somehow, in the patient's unconscious, serves the dynamic function of a mother figure."

Lindemann's[41] reference to the role these key figures play in the psychosocial interactions of the patients points up the other aspect of the kinds of relationships these people have, namely, that they have difficulty in initiating social interaction except through the key figures. This is descriptively recorded in observations that these patients do not readily form friendships, they often are on the periphery of social groups, in which they feel uncomfortable and ill at ease. They may be shy, seclusive, stiff and formal. They may have many acquaintances but few intimate friends. Some feel distinctly estranged from people.

Examination of our own clinical material as well as of case protocols in the literature allows for further delineation of the nature of the key relationships. One gains the impression that the patient lives through the key figure (i.e., mother) and the key figure lives through the patient. Seen from the side of the patient, the patient appears to depend on the key figure as part of his equipment for dealing with the external world. This is revealed by their leaning on the key figure for guidance, advice and direction; by their reluctance to take initiative or to plan independent action; by their tendency to act out wishes, conscious and unconscious, of the key figure, or to live vicariously through the key figure; by their use of this individual as a barrier against or protection from threats from other sources; and by their need to please and placate this figure and to protect her from anxiety or other unpleasant affects, whether provoked by the patient or by others. At the same time the patient's attitude is a highly ambivalent one. The patient is extremely sensitive to, but often frustrated by, the demands of the key figure, but finds it impossible to relinquish his own parasitic relationship. Similarly the demands and requirements of the patient at times are unmet, or only conditionally met, with consequent intensification of the conflict.

In some instances a subordinate figure comes into the relationship. The function of this person is sometimes a buffer one, to absorb the hostility of the patient and of the key figure; sometimes a substitute one, who fulfills much of the same function as the original figure; and sometimes an auxilliary one, to please and gratify the key figure. The choice of spouse is frequently determined along such lines.

Revealing insight into the nature of the object relations comes from observations of the doctor-patient relationship. It is a general experience that the patient either becomes very "dependent" upon the physician or establishes no or at best a very superficial relationship. It is also general experience that patients who develop such a "dependent" relationship, whether or not it is consciously planned by the physician, do better than patients who do not. Of the nine patients who died among my total group of thirty-nine, eight were never able to establish a relationship with me or any other doctor concerned in their care and one had a prolonged relationship (four years) which was unexpectedly interrupted. None of these eight patients was in such critical condition at the time of initial contact that this could account for either the failure to relate or the death. Once a relationship has been established it is unusual for the patient to relinquish it and remain in good health. The patient may leave the doctor because of negative transference conflicts but this is generally accompanied by a relapse, unless a substitute had already been acquired. Any interruption of the doctor-patient contact, especially early in the relationship, is likely to be accompanied by some relapse of symptoms. I have utilized the circumstance of rather frequent one to three day trips out of the city as an opportunity to observe the effects of such enforced separations on hospitalized patients. In general the response is a worsening of symptoms but a denial by the patient that the break in doctor-patient contact was of any importance to him. Eventually, with the doctor's encouragement, the patient may be able to speak of his feelings about such separations and become more tolerant of them. But even under these circumstances it is noted that the patient may use realistic or magical means to maintain a sense of continuous contact with the absent physician. A telephone number and the assurance by the physician that he can be reached in the distant city often suffices, even though the

patient never actually phones. One patient for a whole year carried an unfilled prescription for paregoric, feeling secure in the knowledge that if she could not reach me she had the prescription. Although such situations arose several times, it sufficed for her to think of the prescription to enable her to feel she was in contact with me. Consistent with this is that when the patient has such a relationship, pharmacologically inactive preparations may have striking symptomatic effects, whereas during negative relationships the most potent agents may not only be without effect but may provoke violent symptoms.[21,22]

Mothers

There is also an impressive consistency in the description of the mothers of patients with ulcerative colitis, although there are differences in how the women describe their mothers as compared to how the men do. (See references 5, 6*a*, 9, 10, 13, 15–17, 22, 25, 28, 31, 34, 37, 38, 43, 44, 46, 50, 51, 55, 57–59, 65, 67, 69.) It is important to emphasize that the bulk of data on the parents comes from patients' descriptions; there are no systematic or direct studies of parents of patients with ulcerative colitis, although some information is available from those who have worked with children.[28,57,58,59,66]

In general, the mothers are described as controlling and dominating. The women are more likely to think of their mothers as powerful and overwhelming figures, who make them feel helpless and dependent. They often describe their mothers as cold, unaffectionate, punitive, rigid, strict and judgmental. The men, although describing similar domination, are much more likely to find it acceptable and to describe their mothers as kind, considerate women who worried constantly about their well-being. Despite these differences in attitudes of the men and women toward their mothers, one readily finds many similarities among the mothers. In general they are either unhappy, pleasureless, gloomy women, with no great zest or enjoyment in life, or hard driving perfectionistic women who are constantly dissatisfied with their own or others' accomplishments. They tend to be worrisome, complaining, pessimistic, and often hypochondriacal. A high proportion show moderate to severe obsessive-compulsive traits, a smaller proportion show pathologically disorderly behavior or eccentric preoccupation with collections of odds and ends. A few are psychotic characters or frankly psychotic, usually paranoid. Included among the latter are religious zealots with powerful feelings of right and wrong and hell and damnation, food faddists and self-righteous, self-appointed guardians of public morals. Many mothers are described as having depressions.

A prominent feature, described in different ways by many patients, is the mother's propensity to assume the role of a martyr. This includes the mother (usually described by a male patient) who devotes herself selflessly to everyone, sacrifices herself, suffers humiliation and hardship, all for the health and welfare of the patient; the mother who displays and demonstrates her martyrdom to her family, mobilizing guilty reactions from the patient; and the mother who approaches religious and social martyrdom in her public display. One is left with the clear impression that the majority of the mothers are masochistic characters and that the relationship between mother and patient is very much determined by this. The patients appear to be extraordinarily "in tune" with the shifting dynamics of the mother's needs, anxiety and guilt. This is particularly evident in the response to non-verbal cues. Many patients comment on their sensitivity to mother's sigh, look or change in posture or facial expression. Some patients, particularly the men, submit passively and obediently to the mother's domination, while others, also submitting, do so with the complaint that mother won't permit them to do otherwise.

Several authors[6a,14–17,25,66] have been impressed with the unconscious gain to these mothers to have their children sick. Not only does it seem to assure complete dependence and submission but also an opportunity for the gratification of certain sadomasochistic needs. The reciprocal gain for the sick patient is obvious. This is well illustrated by the statement of a twenty-two year old woman about her mother: "She always had to inspect and regulate, so that when I became sick it was very gratifying to her. She took pleasure in the diet and was completely preoccupied with doing the right thing. She penetrated into all levels of control, even to regulate my bowel movements. Her health was never better than at that time." It is notable how many mothers insist on taking care of their sick adult sons or daughters even when there are others to do it.

The quotation just cited also tells something

of the nature of the control sought by the mother and by the patient. Sperling[65–67] uses the term "emotional symbiosis" to characterize this relationship. Some patients in analysis have actually verbalized that they feel like a physical appendage to mother, as if an organic unity existed. In many spheres of living they literally abdicate any sense of individuality and personal participation and act as a proxy for the mother, enjoying only what she would enjoy and forever seeking assurance that no transgression had taken place. This perhaps is responsible for the common description by patients of their mothers as "unpredictable." It is the patient who needs constantly to know what mother's needs and wishes are at every moment and any unanticipated behavior is felt by the patient as inconsistent, quixotic, impulsive and dangerous. Yet there is evidence to indicate that some of the mothers, certainly the psychotics and psychotic characters, truly were unpredictable and inconsistent to a high degree. In any event the patients' technic to deal with mother is markedly colored by attempts to anticipate and fulfil mother's wishes, to alleviate mother's anxiety and guilt, and to manipulate other people and situations so that even outside factors will not upset mother. In other words, he "learns" the conditions under which he will not be rejected.

Comment: It is again necessary to emphasize that the bulk of the information about the mothers comes from the patients. It is difficult to know whether the consistency in the descriptions of the mothers and of the mother-patient relationship is a reflection of the common psychology of the ulcerative colitis patients or whether it truly indicates a close similarity of these mothers. I am inclined to believe that both factors play a role. Sperling,[65,67] Prugh[57,58] and Gerard,[28] who have studied children and hence have made direct observations on the mothers, described them as rigid, inconsistent, at times overindulgent, at times overwhelmingly dominating, extremely ambivalent and lacking in maternal qualities. Sperling[66] in particular stressed strong unconscious destructive impulses toward the child and her need to keep the child in life-long dependency for the gratification of vitally important bodily and emotional needs. Gerard emphasized also the mother's ambitiousness for her child's achievement, pushing the child for independent but conforming behavior. The one mother I have studied directly was an extremely perfectionistic compulsive woman who warded off anxiety by demanding constant achievement from herself and her family, but especially from her oldest son, who developed colitis. Nonetheless, analytic observations of patients with ulcerative colitis leave little question that many of the characteristics ascribed to the mothers have been contributed to quantitatively, if not qualitatively, by the patients' own projections.

The descriptions of the mothers and of the mother-patient relationships clearly provide a basis for the characteristic relationships already described. The "key" relationship usually is with the mother or a mother surrogate.

Fathers

It is remarkable how much less information is available about the fathers than the mothers. Many protocols barely mention the father and some do not refer to him at all. Among my patients in analytic psychotherapy far more time was spent by the patients on material about the mother. In many instances only a very shadowy, stereotyped picture of father was obtained, and that chiefly on direct inquiry. I believe the sparsity of information about the father is a reflection of the lesser role played by the father in the development of these subjects.

In our material as well as in the literature there is some difference in the descriptions of the fathers of the women as compared to the men. (See references 2, 6*a*, 9, 10, 13, 25, 31, 34, 35, 38, 43, 44, 55, 69, 78.) In general women are more inclined to present their fathers as gentle, kind, passive, usually ineffective men to whom they are quite attached, while the men are more likely to describe father as brutal, punitive, threatening, coarse and very masculine. There are a number of exceptions on both sides. Thus some of the women's initial descriptions later changed to indicate a more aggressive, rigid, punitive man. That the relationship with father also had sadomasochistic overtones is revealed by the complaints of some women, usually later in therapy, that the father was too lenient. Men patients tended to see the fathers as threatening and abusive to the mother, who suffered thereby, and with whom the patient identified. The man often seemed to feel that the father regarded him as a "sissy." The women, on the other hand, often complained that the father did not adequately protect them from mother's aggression. The father was particularly likely to be described

in positive terms when he had died while the patient was still a child.

Objective data on the fathers are not available and the impression of any consistent pattern is not to be found. Among them we find alcoholics, passive inhibited characters, psychotics (usually paranoid schizophrenics), eccentrics and obsessive-compulsive characters. We cannot support Brody's[6a] statement that the fathers are usually healthy men. The important finding is that the father is so often so inconspicuous in the patients' accounts.

Siblings

Of note here is only that the siblings are so often described as more outgoing and uninhibited (even when they are not) and as having a favored status with one parent. Often the complaint by women patients is that a brother is favored by mother even when he is not so considerate of mother as the patient. Men patients may say a brother is more accepted by father because he is more masculine.

Sexual and Marital Adjustment

All data available support the conclusion that the sexual development of these patients is inadequate. (See references 2, 7, 8, 11, 13, 15–17 25, 31, 34–38, 43, 47, 50, 51, 55, 59, 64, 65, 67, 69–71, 78 and 80.) In terms of overt behavior, interest and participation in sexual activity is low. Most of the women are frigid and even those who experience orgasm do so but rarely. A good number of men and women remain with little or no heterosexual experience even when married. The men who are not impotent have a rather desultory interest in heterosexual activity. The man often assumes a passive role in relation to his wife, who may be the one to initiate sexual activity. One sixty-one year old man had intercourse with his wife only three or four times in more than thirty years of married life, all within the first year. I have not yet seen or read of a man or a woman with ulcerative colitis who had enjoyed a vigorous, hearty, active sexual life. Many patients acknowledged a wish to be fondled or cuddled, really like a child, but at the same time were totally rejecting any genital approach.

They are prone to regard sexual activity in anal terms. They use terms as dirty, soiling, digusting, unclean, etc., and they feel squeamish about body contact, secretions and odors. Many feel obliged to bathe immediately after a sexual contact. Rigid moral standards are common. Such attitudes often reflect the mother's attitude. Ascetic rationalizations are not infrequent. Latent homosexual trends are apparent among some of the men but I have not yet seen or read of an overt homosexual who developed ulcerative colitis.

Many patients with cecostomies or ileostomies are observed to derive erotic gratification from manipulation of the stump.[25,45,46,64] In some instances, patients replace genital masturbation with manipulation of the stump, which shows tumescent responses, and then enjoy conscious sexual fantasies, sometimes homosexual. Other patients show an intense preoccupation with cleaning, wiping and manipulating the stumps, but without admission of conscious sexual feelings. It is not yet known whether this erotization of the artificial opening is more prominent among the ulcerative colitis patients than among others.

The marital rate is probably not different among this group of patients than it is in the general population, although actual figures are not available. Among the thirty patients over twenty-five years old in our series, all but four were or had been married. In most instances the characteristics of the marital relationships could be predicted from the nature of the object relationships and sexual adjustment, which have already been discussed. In some instances the husband or wife really fulfilled the role of a succoring, sustaining mother. More often the spouse took a subordinate role to the mother and occasionally was the latter's conscious or unconscious choice. Not infrequently it was the mother-in-law, who closely resembled the mother, who was the real object, the spouse being treated more like a sibling.

Pre-morbid Bowel History

Although many writers have speculated on the significance of early bowel patterns and training experiences among these patients, there actually is remarkably little information on this point. Elsewhere we have reported that a high proportion of our patients suffered from constipation prior to the development of the colitis and that this often had its inception in childhood.[19,20] No reliable information is available as to the age and character of toilet training of these patients. Gerard,[28] reporting on eight cases among children, states that in all cases bowel training had been early and punitive. Prugh's[59] studies

indicate that while early and coercive bowel training was common among patients with ulcerative colitis, it was not a major factor in the absence of associated personality disorder. Lindemann[42] found a confirmed history of severe toilet training in only two of forty-five adults but many factors make the securing of an accurate history difficult.

Childhood material indicative of intense conflictful relations with the parents regarding bowel activity is prominent in the histories of some patients and totally absent in others. Thus some patients tell of the mother's or father's intense interest in and control over their child's bowel movements, the punitive or seductive administration of cathartics or enemas, coprophilic and soiling acts as a child, bowel activity in response to parental pressure or conflict. (See references 2, 7, 13, 25, 31, 32, 50, 51, 57–59, 70, 71.) Yet more published protocols make no mention of such material and some patients emphatically deny their occurrence. It is perhaps significant, however, that among our patients the longer the patient was in therapy, the more likely was such material encountered. Nonetheless, it cannot be said at this time that the data available indicate a very intimate relationship between overt childhood excretory behavior and the occurrence of ulcerative colitis. Different kinds of data are necessary to settle this point.

Precipitating Circumstances

The identification of the somatic processes which are pathognomonic of ulcerative colitis makes it possible to establish more precisely the exact time of onset of the disease in many cases and thereby to examine the circumstances under which the disease becomes manifest. Elsewhere we have pointed out that the presence of blood in the stools is the most common initial symptom, often preceding diarrhea.[19,20] This is generally true when the disease begins in the distal left colon, the most common initial locus. When the process initiates in the right or transverse colon, diarrhea and cramps without gross blood are usually the first symptoms. In establishing exactly the onset of the disease it is necessary to inquire into the first clear deviations from usual bowel activity. Many patients are found to have had rectal bleeding or abrupt severe constipation for days, weeks or even months before diarrhea begins. When the onset of disease is so established, we find that the time interval between a psychologically stressful circumstance and the onset of the first symptom of the colitis often is a matter of hours or a day or two. On the other hand there are cases in which the onset is rather gradual and not easily timed and in which one deals not with a well defined stressful experience but rather with a gradually changing psychic status, during which symptoms gradually and sometimes intermittently develop. (See references 2, 7–11, 13–15, 19, 20, 31, 35, 37, 38, 41, 44, 57, 58, 64, 66, 70, 71, 78.)

In our series the onset was precisely established in thirty patients who were also studied psychologically. The following kinds of onsets were observed.[19] Ten patients noted blood as the first symptom, with diarrhea coming on later or not at all. In five of these the onset was associated with a discrete event concerning which the patient was consciously disturbed and the bleeding followed within days. In one case there was an abrupt onset of bleeding following a New Year's party, but the significance of this was not elucidated. The bleeding began gradually in a girl during adolescence, and in a thirty-one year old man during his wife's first pregnancy. In two adults the first episodes of bleeding occurred in childhood and no memories of the circumstances at the time of onset were available.

CASE REPORTS

CASE 1. A thirty-one year old woman became pregnant a few months after the birth of her first baby. Two years earlier, when she discovered her husband was having an affair, she separated from him and returned to her mother. After six months a reconciliation was accomplished, although the mother was pessimistic of its outcome. The patient decided that having a baby would help hold her husband and the first child was conceived in this setting. When she found herself again pregnant a few months later, rectal bleeding began. Stools remained constipated and bleeding continued for six months and then diarrhea developed. The patient thought that a second pregnancy so soon was too much and that her husband might again leave her.

In seven patients ulcerative colitis began with bloody diarrhea. Three of these showed an abrupt onset in settings in which the patients were consciously disturbed. Four of them began during or just before adolescence (ages twelve

to sixteen); in one boy this began soon after the death of his mother and in one girl while the father was depressed.

CASE II. *Bloody diarrhea:* A twenty-nine year old woman married when she discovered she was two months' pregnant. She hoped to hide the premarital conception from her puritannical mother by saying the baby was born prematurely. Gestation actually was seven months so the baby was born five months after the marriage. Two days after the baby was brought home and her mother arrived to help the patient had abrupt onset of chills, fever and diarrhea which became grossly bloody in a few days.

Five cases began with abrupt and severe constipation, usually associated with severe rectal or lower abdominal pain, and followed in a few days by bloody diarrhea. In four of these patients this followed within hours or days of an upsetting event. No clear-cut precipitating event could be elicited in one interview in the fifth case.

CASE III. *Acute constipation:* A twenty-one year old married woman was awaiting the return home of her soldier husband, whose train reached the city that day. After keeping her waiting several hours while he visited his mother, he appeared at the door, and without further elaboration announced that he wished a divorce. On this note he left. The same day she was seized with terrific cramp-like pain in the left lower quadrant, an urge to defecate, but inability to do so. She was admitted to a hospital where she was given eight enemas in two days before any relief was achieved. Following this she had formed stools, three to four times a day, for a month, and then small amounts of blood were first noted. Thereafter she passed blood and mucus four to five times a day, stools became semiformed, and then grossly diarrheal and bloody.

Four cases began with bloodless diarrhea of fairly abrupt onset. In a thirteen year old boy diarrhea developed just before his bar mitzvah, an event which he prepared for under great pressure from his mother. In three no discrete precipitating event was disclosed but rather a change in over-all psychologic adjustment. One patient lost her father and then over the next ten months felt her relationship with mother threatened by shifting alignments in the family. A man took over his father's business at a time that his wife was pregnant for the second time. A third, after many years of attempting to cope with a psychotic mother, herself began to feel estranged and depressed, then developed diarrhea and fever, with a feeling of "relief" to be sick. In all three cases the patients believed they were obliged to accomplish more than they were able to do.

Four patients showed very mild diarrhea or intermittent bouts of diarrhea for several months or years before ulcerative colitis was finally diagnosed. In three this began first before or during adolescence.

CASE IV. *Insidious diarrhea:* A fifteen year old girl noted over a period of two months a gradual increase in the frequency of her bowel movements which, however, remained formed but soft. During this time there was increasing turmoil over her changing status as an adolescent. She was then in an automobile accident, which involved no serious injury but did represent a disastrous outcome of an attempt to identify with her adolescent peers. Immediately after the accident her bowel movements became watery and frankly bloody.

The case reports in the literature and our own data indicate clearly that the mucosal reaction may occur within hours or days of events which abruptly upset a precarious psychic balance or more gradually during periods of changing psychic equilibrium. While such a close time relationship between the disturbing events and the onset of symptoms strongly suggests a relationship between the two, the interpretation of the meaning of the "precipitating" event presents more difficulty. For convenience I will consider first the cases in which the event was distinct, with the onset promptly thereafter, and then the cases in which the disease began over a more gradual period of psychologic change.

In my series there were sixteen cases with acute onset in which well defined events immediately preceded the bleeding. Eleven began with bleeding, with and without diarrhea; four with acute obstipation followed by bleeding; and one with diarrhea without gross blood. Summarizing the data, the following phenomena were noted: (1) real, phantasied or threatened interruption of a key relationship; (2) demands for performance which the patient felt incapable of fulfilling, especially when support had been withdrawn or when disapproved activities were involved; (3) overwhelming threat from or disapproval by a parental (usually) figure. Common to all these circumstances was that the patient acutely felt helpless to cope with what

was happening and previous psychologic defenses, such as compulsive mechanisms or magical thinking, crumpled.

The cases in the literature with acute onset follow closely the same pattern. Murray stressed sexual problems, which are indeed prominent among many patients, but the immediate precipitating events were ones which overwhelmed the patient with helpless despair.[50,51] Groen stressed that the precipitating situation in his six cases, all of whom had an acute onset, was one with which the patient could not cope, involving an acute love loss and a painful humiliation.[35] He stressed the helplessness of the patient. In all of his cases an actual or threatened separation occurred. In two of the cases an intensification of compulsive and phobic symptoms preceded the final break. The cases of Jackson,[37] Karush,[38] Cobb[9] and Small[64] began after separations. Grace's two cases of bleeding and one of diarrhea began soon after separations while the other case of diarrhea began when the patient's attempts to achieve a response from his mother by gifts and performance failed.[31] Prugh's nine year old patient bled after being ousted from his mother's bed by his returning soldier father.[57,58]

In the cases beginning insidiously, usually with slight bleeding or mild intermittent diarrhea, one usually deals not with a clear-cut precipitating event but rather with a period during which mounting pressures threatened previous psychologic compensation. In many cases, after months or even years of such mild and intermittent symptoms some event acted as "the straw that broke the camel's back," precipitating the full blown syndrome. Ten of our cases fit into such a group. Four began during adolescence. Two men and two women developed symptoms during periods of mounting responsibilities associated with marriage and the birth of children, while relations with supporting parental figures were disturbed, and two became ill during severe psychiatric illnesses of parents. The cases in the literature also follow this general pattern.[8,31,38]

Comment: The significance of the precipitating events or circumstances begins to have meaning in terms of the processes already discussed. At the risk of a premature and oversimplified generalization, but in order to focus attention on problems that will require further clarification, I suggest the following factors to be necessary, but not sufficient, conditions for the development of the somatic condition. (1) The important interpersonal relationship (or relationships) which has been an integral part of the patient's total adjustive process becomes less effective. (2) The pressure from instinctual forces, all at a pregenital level, becomes relatively or absolutely (e.g. in adolescence) stronger. (3) The character defenses, already described, become inappropriate or inadequate under the circumstances. (4) The patient is increasingly thrown on his own resources for performance. It is to be noted that these four factors are mutually interdependent. Other investigators have stressed one or another of these aspects of the dynamics of the precipitating situation. Groen stressed the acute love loss and a painful humiliation.[34,35] Grace spoke of the importance of symbolic rejections or a threat to a dependent position.[31] Paully spoke of the precipitating circumstance as anything threatening the patient's dependence.[55] Daniels described precipitating situations as those in which there is a serious threat to security.[15] Lindemann laid major emphasis on loss of security, especially loss of another person of emotional significance, a sudden decrease in rate of interaction.[41] Among his eighty-seven cases, seventy-five occurred after a bereavement, two occurred after loss of cultural milieu, six occurred with threat of psychoses and four occurred after hemorrhoidectomy. Unfortunately, these interesting data do not include information as to when the alleged precipitating event occurred in relation to the onset or what the presenting symptoms were. Lindemann lays great importance on a "delayed grief reaction." Among thirty-one of our patients fifteen might be considered to have unresolved grief, ten due to deaths. Alexander emphasizes the general psychodynamics of the excremental function as conspicuous in the precipitation of the disease.[1] He states that the first symptoms frequently appear when the patient is facing a life situation which requires some outstanding accomplishment for which he feels unprepared. Karush and Daniels state that the attack began soon after a sudden, unforeseen threat to the security of a precariously effective adaptive system and that, in the face of an emotional blow, there was loss of successful mastery and depression.[38]

It is clear that these points of view are in essential agreement but differ in emphasis. In order to get further insight into the pathogenic process, I propose now to examine the conditions under which relapses occur.

Relapses

Rigid criteria must be established as to what constitutes a relapse. Following the characterization of the somatic process already discussed,[19,20] relapses, for the purpose of this analysis, are defined as *episodes that begin with bleeding after a period of normal bowel activity of a month or more.* Even though some episodes of diarrhea without bleeding and diarrhea that later became bloody obviously are relapses, they will not be included because other determinants, including anatomic factors, may be involved in the genesis of the diarrhea and cannot be clarified by the kind of information available to us.

Using such criteria we have data on thirty relapses among ten patients. One patient, observed over five years, experienced seventeen bleeding episodes, most of them mild and short-lived. Twenty-four episodes involved real, threatened or symbolic separations or anniversaries of such. For all of these some behavior of the patient or some external event seriously reduced or threatened to reduce the effectiveness to the patient of a relationship and as a consequence the patient actually or in fantasy felt a loss and under pressure to assume more independence. In all of these instances the patient felt unable to do so and in this setting the bleeding began. The six exceptions, not directly involving separations, provide interesting confirmation. In all the patient found himself in a situation in which he felt endangered or enraged but felt powerless to do anything. The common denominator to all the bleeding episodes appears to be a feeling of relative helplessness.

It is difficult to evaluate relapses in the literature from this point of view since most protocols do not provide sufficient data either as to symptomatology or as to the circumstances. However, I consider the following reports to include examples which might be regarded as confirmatory of my data: references 35, 31, 2, 78, 14, 47, 65, 66, 38.

Affective Status

The data on relapses immediately focus attention on the affect experienced by the patient at the time of onset and during relapses. For present purposes we are dealing with the affect which enters consciousness, recognizing that this may not be the original one but a multi-step elaboration. The identification of the affective status is made from direct observation of the patient's behavior, from the verbal content during interviews or therapeutic sessions, and from the patient's own description of how he felt. Data are available on affect during forty-five onsets and relapses among sixteen patients. In all instances the feeling tone at the time of symptoms development was designated by such expressions as "helpless," "despair," "hopeless," "overwhelmed," "too much to cope with," "too much to expect of me." In general there was a fairly good correlation between the severity of the symptoms and the degree of helplessness or hopelessness felt by the patient. In one case this affect was covered by the opposite behavior; the patient intensified his activity and work and for several weeks denied both symptoms and affect. When he could no longer deny the presence of bleeding, he was plunged into an abyss of despair and bleeding became massive. In many instances these feelings merged into depression, with added qualities of being alone, bereft, deserted, unwanted, unworthy. It was notable that conscious anger, even if inhibited or denied, did not precipitate an attack unless it was followed by a feeling of helplessness and despair.[21]

Examination of the literature reveals no systematic attention to the affect preceding the onset or relapse of ulcerative colitis. None of the writers has made an adequate distinction between relapses and the reactions of the chronically diseased bowel. Thus it is difficult to evaluate either their interpretations or their protocols in terms of identifying whether any affect was consistently associated with the onset of the disease or with relapses as I have defined them. Lindemann mentions helplessness, sorrow and depressions as the emotions associated with attacks.[41,42] Groen defines the affect as humiliation and defeat.[35] The protocols of his cases include such descriptive expressions as "disappointed," "lonely," "humiliated," "miserable," "absolutely crushed," "powerless," "shocked," "hurt" as preceding episodes of bleeding. West describes his patient's "inability to secure himself against injustice."[78] Sperling notes the extreme helplessness experienced by the two children she studied.[66] Grace says the attacks occur in settings of anxiety and resentment, when the patient feels frustrated, humiliated, angry and resentful. Their protocols indicate helpless, despairing attitudes.[32] One of Appel and Rosen's cases felt "humiliated" when his sisters left.[2] Cushing noted "bowel symptoms"

to develop when control was threatened.[13] Karush and Daniels noted in one of their analyzed patients that when she was "overwhelmed by fear, hopelessness and despair," bowel bleeding or diarrhea would follow.[38]

Some writers stress "fear," "anxiety" and "apprehension"[43,50,51,69] while others emphasize "rage," "resentment," "anger"[55,57–59,64–67] but none of these terms is sufficiently supported by the data presented to allow for critical comment.

Comment: The discovery that the episodes of bleeding are consistently associated with a feeling of helplessness or despair is an arresting observation but before any conclusions are drawn I propose first to examine what other psychosomatic disturbances are manifested by these patients as symptoms alternative to bowel symptoms and what affect, if any, is associated with such symptoms.

Alternative Symptoms and Associated Affect

Headache. By far the most common alternative symptom experienced by patients with ulcerative colitis is headache, although this seems to have escaped the attention of most observers.[21] Among twenty-three patients in whom adequate histories were obtained twenty suffered from headaches. In fifteen the headaches were severe enough to have required medical attention. Seven patients had headaches as a major symptom before ulcerative colitis developed. In general, headaches and active colitis did not coincide. Headaches either antedated the onset or occurred during intervals when the patient was relatively free of colitis. Thus of fifty-six headaches only five occurred during a period when the patient was having bleeding or diarrhea. The occurrence of a headache during an attack of colitis was often found to herald the end of the attack.

Ten patients had headaches which clinically appeared consistent with the diagnosis of migraine. Ten patients had muscle tension or hysterical conversion type headaches. Some of the patients who had migraine also had other types of headaches.

Fifty-six headache periods were observed during the therapy of nine patients. The two patients longest in therapy accounted for forty of these. The headache periods presented a striking contrast to the periods of active colitis. On forty-six occasions it was found that the headaches occurred when the patients felt in control, had taken an active stand, made a decision, thought something through, but that this was followed by conscious or unconscious guilt. In none of these instances did the patient feel helpless or afraid. In all of these examples there was evidence of strong conscious or unconscious aggressive or sadistic impulses. In many the patients spoke of the intensity of the thinking process associated with contemplated action. Other observers, including family, doctors and nurses, frequently commented on the unusual "aggressiveness" or "assertiveness" of the patients around the time that the headache occurred. Occasionally the headache occurred on the anniversary of or in response to something symbolic of some previous occasion when the patient felt self-assertive or aggressive.

Four headaches (all of one patient) were not so clearly associated with such identifiable feelings but rather with an identification with the father, who also had headaches. Her headaches were considered to be hysterical and it is perhaps significant that at other times the patient suffered from other hysterical pains. Five headaches did not fit into any identifiable pattern.

Only one headache (of fifty-six) occurred when the patient felt helpless, sad, "unable to solve all these things," wondering if I was trying to get rid of her. Interestingly, she also had diarrhea and anorexia at the same time.

When directly queried, patients were unequivocal in contrasting the headache and the colitis status. They all contrasted the active character of their thinking and behavior before the headaches to the passive, helpless attitudes before the colitis attacks. Conscious and unconscious selfpunitive attitudes were common in association with the headaches.

The literature contains no reference to the occurrence of headaches among patients with ulcerative colitis and I am able to find only three reports which incidently mention headache.[8,38,40] Although Karush associated the headaches "with fluctuations in the intensity of the erotic feelings toward the analyst" there is insufficient information in the protocol to verify the interpretation.[38] It is significant that the headaches occurred in a period when his patient had no bowel symptoms and was more quickly mobilizing and expressing rage at frustrating figures. Furmanski, in a study of 100 patients with migraine, found "colitis" in eighteen (ulcerative colitis in one, mucous colitis in seventeen).[27] Among sixteen patients with mucous or spastic

colitis in my practice, twelve had headaches. Kepecs describes a patient who had diarrhea when compliant, submissive and unable to express anger, and migraine when aggressivity became overt and expressed.[39]

Comment: These data are of interest as indicating different levels of psychologic function corresponding to the two kinds of somatic symptoms. It renders untenable the concept that ulcerative colitis is simply the way in which these individuals respond to an emotional stress. Rather it indicates that a particular psychobiologic situation is a prerequisite for the development of the bowel disorder. So far, the consistently found affective status of "helplessness" provides the best clue as to what these psychobiologic requirements are. The significance of headache among these patients is the subject of a more detailed report elsewhere.[21]

Upper Gastrointestinal Disturbances. Anorexia, nausea and vomiting commonly accompany attacks of colitis (and may also accompany the headaches). But some patients suffer from such symptoms independently of the colitis. Ten of twenty-three patients had such a history and three dated the symptoms from childhood. Since none of these patients had these symptoms independently of colitis during my observation I have no precise data as to the psychic determinants. In one adolescent boy the nausea and vomiting appeared to be hysterical conversion symptoms. Two women, twenty-five and eight years before the colitis, each had a period of extreme anorexia and weight loss, clinically resembling anorexia nervosa, but in both cases this information was only obtained from other sources (old chart and family) and was not revealed by the patient.

One woman gave a history of a peptic ulcer before the colitis, said to be confirmed radiologically, and one man had symptoms suggestive of ulcer but none was demonstrated by radiologic examination.

The literature contains very little information. One of Grace's[31] patients had frequent vomiting in childhood, and Szasz[72] reports one patient who also had a peptic ulcer. In the latter patient the two syndromes never occurred at the same time.

Skin. One patient had eczema in childhood, one had urticaria, one had generalized pruritus, and one pruritus ani—all before the first attack of colitis.

Mahoney's[43] patient had severe eczema, Karush's[38] patient developed angioneurotic edema during analysis, and Kubie's[40] patient had an episode of severe generalized dermatitis. Sperling's[66] patient developed a leg ulcer.

Miscellaneous Manifestations. Among my patients the following manifestations were observed; one patient had hypertension, Ménière's syndrome, facial tic and hayfever; four patients had ulcers in the mouth; one patient stuttered, one had hyperventilation syndrome, another arthritis, two had globus, one showed accident proneness and back pain, and one had had rheumatic fever.

The literature adds examples of toxemia of pregnancy,[70] paroxysmal tachycardia,[14] asthma,[14] ureteral spasm,[13] vasodepressor syncope,[13] obesity,[43] rheumatic fever,[38] thyrotoxicosis[38] and leg ulcer.[66] While all these presumably represent random, incidental disturbances, experience with headaches, the frequency of which escaped my attention as well as that of other observers, leads me to believe that future study should give greater attention to the true incidence of these other disturbances as well as to the circumstances under which they develop.

Gross Psychopathology

Clinical Depression. I have already commented on the fact that some degree of depression is generally evident during active colitis. Alternation of mood from depression to mild elation commonly correlates with the magnitude of bowel symptoms and many patients described such mood swings to precede the onset of colitis. Grace et al. disagree with this and state that "subjects with ulcerative colitis may become constipated when depressed" and "it is well recognized that subjects with ulcerative colitis who enter into a period of psychotic depression lose their symptoms of bloody diarrhea."[31] They cite the case of a man whose ulcerative colitis became quiescent when he became seriously depressed but bring no additional clinical evidence in support of the statement.

Two psychotic depressions were observed among my patients. Both occurred simultaneously with a relapse of colitis. One patient was given electric shock treatment and both the depression and colitis disappeared. The second patient died from massive hemorrhage before any treatment could be instituted. The patients of Cobb and of Tunis also had active colitis and

psychotic depression simultaneously.[9,74] Daniels[14] and Lindemann[41,42] also discuss depression as associated with colitis.

Paranoid Disorders. There is general agreement among those who have discussed the problem that delusional and projective mechanisms are the most common features of the psychotic developments in ulcerative colitis.[1,2,14,38,41,42,61,66] The incidence, however, remains unknown. Moses reports an incidence of 20 per cent among patients in analysis.[49] Among my patients, two had acute psychotic episodes occurring some years after the first attack of colitis. Both patients settled into more or less chronic paranoid states, with well organized delusions, and continued to have attacks of colitis and of headaches. It could not be established what, if any, bowel symptoms were present during the acute psychotic periods. One patient, during adolescence and many years before the colitis developed, had the persistent delusion that she had a bad odor. Another patient, during analysis, had several brief periods when she had the idea that her mother-in-law or her husband was trying to poison her but she always was able to convince herself of the unreality of these ideas.

Although there is a general impression in the literature that psychosomatic disturbances and psychotic manifestations have a reciprocal relationship, this is by no means demonstrated in ulcerative colitis.[2,40,61,66,71a] My two patients as well as the two patients reported by Appel and Rosen,[2] and one patient each of Sullivan[70] and Wittkower,[80] showed psychotic behavior and active colitis at the same time. While it is true that in these cases the psychotic behavior seemed to be less manifest during the more severe colitis symptoms, much longer periods of observation are necessary to clarify the relationship between the two processes.

Neuroses. I have already discussed the characterologic features of these patients. The prominence of obsessive-compulsive character traits was mentioned. Yet no patient in my series presented a full-blown obsessive-compulsive neurosis. Only one of Wittkower's forty patients was so described, although fourteen had obsessional thoughts.[80]

Conversion and phobic symptoms are occasionally noted but certainly are not prominent.[14,80] One of my patients had hysterical pain and one of Daniels'[14] patients had hysterical amnesia, pain and phobic symptoms.

Ulcerative Ileitis after Ileostomy

Elsewhere I have discussed the development of a pathologically similar lesion in the ileum of patients with ulcerative colitis.[19,20] In the present series ulcerative ileitis developed in five patients. In three the ulcerative ileitis developed after ileostomy and in bowel which had been demonstrated to be normal at the time of ileostomy. In all three the precipitating circumstances and the affective status were identical with that observed during previous attacks of ulcerative colitis. In the other two patients the time of onset of the ulcerative ileitis could not be established.

There is no reference in the literature to the conditions under which ulcerative ileitis develops. Regional ileitis is a different disorder.[75a]

CORRELATIVE STUDIES

These are of three types. The first is the reporting of occasions when some bowel symptom occurs during observation of the patient and an attempt is made to relate the simultaneous psychologic data to the bowel symptom. The second is the simultaneous psychologic and physiologic study of patients with fistulas. The third is the correlation of symptomatology with psychic status in the course of observations during psychoanalysis and long-term psychotherapy.

Correlation of Symptom and Psychologic Data. Such short-term random observations have been reported by Murray,[50,51] Sullivan,[70,71] Daniels,[14] Groen,[35] Prugh[57,58] and Sperling.[66] They are of dubious value in ulcerative colitis. My experience is that patients do not report with any consistency symptoms experienced in the presence of the investigator. At times the act of announcing or displaying the symptom may be more significant behavior than the symptom itself. Further, when patients are having frequent bowel movements, the coincidence of a cramp or bowel movement in the examiner's presence cannot be interpreted, no matter how dramatic the psychologic material may be, unless something is known of the frequency and distribution of such symptoms over relatively long periods of time.

Fistula Studies. Study of patients with colonic stoma permits simultaneous observation of changes in the bowel and psychologic processes.[32,45,77,79a] Three patients with ulcerative colitis have been studied in this fashion. The significance of these studies for an understanding of the nature of the somatic process has been critically discussed elsewhere.[20] The present

discussion will be confined to an evaluation of the attempts to correlate the psychologic data with the changes in the colon.

Grace studied four fistulous subjects, two with ulcerative colitis.[32] This is a valuable work containing much useful information but it is not without limitations, due mainly to deficiencies in the design of the study and to the way in which the data are presented. Their conclusions are summarized as follows: "Overwhelming life situations provocative of abject fear and dejection were associated with hypofunction of most of the large intestine with pallor, relaxation, lack of contractile activity, and relatively low concentrations of lysozyme in the colonic secretions Life situations provocative of conflict with feelings of anger, resentment, and hostility or of anxiety and apprehension were found to be associated with hyperfunction of the colon . . ." Sustained hyperfunction was thought to lead to increased fragility of the mucosa and eventually to the changes of ulcerative colitis.

These conclusions are somewhat at variance with what I have just summarized in this report. However, I believe the discrepancies are more apparent than real and result mainly from an overinterpretation of the data. A serious weakness of this study, which significantly limits the conclusions which can be drawn from it, lies in the handling of the psychologic material. Unfortunately the authors do not establish clearly and set down in print what criteria they used for the various psychologic categories (e.g. abject fear, anger, resentment, apprehension, etc.) which are correlated with the physiologic states. This omission is compounded by an extremely meagre reporting of primary psychologic data, leaving the reader without means of examining their interpretations. Thus the important conclusion that "abject fear and dejection" lead to hypofunction of the colon (which is contrary to what would be predicted from the data of this review) actually is supported only by two brief sections[32] (pp. 93, 154) which describe the patient as "mildly depressed" for four weeks. It is said that the intensity of the "depression" varied from day to day and that the colon was most inactive when "hopelessness was most pronounced," but no further documentary data are given as to the nature and dynamics of this state. Further, no information is available as to the dynamics of the doctor-patient relationship at this time. This is an important omission throughout the study. Nowhere is adequate attention given to the significance of the experimental situation as a determinant of this relationship. Much of the material points to the likelihood that submission by the patient to the demands of the research had become a condition, mutually operative, for maintenance of the doctor-patient relationship. This seems most evident in the stress interviews and the painful procedures, in which the patient was angry or anxious, but *helpless and unable to refuse further participation*, lest the relationship with the doctor be jeopardized. It is perhaps significant that one patient without colitis who objected vigorously to the testing of pain response and who refused to have the experiment repeated (in contrast to the colitis patient) showed hypofunction of the colon as a response. Clearly both patients had conflict, but the conflict was handled differently. Indeed, as one examines the available but limited data on the hyperfunction responses it seems likely that the "resentment" or "anxiety," which remained largely unexpressed, occurred in settings in which important relationships, including that with the experimenter, were believed by the patient to be in jeopardy. Most of the protocols also speak of humiliation, embarrassment, feelings of being ignored and neglected, ("who will take care of me?") as associated with what the authors have described as resentment or apprehension.

All this suggests a greater concordance with the anamnestic data reported in this review and points up the importance of complete reporting of primary data if unique studies of this type are to have the lasting scientific value they deserve.

One of the patients reported in the Grace study (Subject C) was also studied by Margolin[45] and Winkelstein[79a] but no data are available. Only the general statement is made that when an outward display of "emotions" was suppressed the mucous membrane "was seen to flush a fiery red and minute petechial mucosal hemorrhages appeared."

Wener and Polonsky studied a twenty-one year old woman with ulcerative colitis who had a transverse colostomy.[77] Their technic of study was essentially the same as that of Grace, and as such is subject to much the same criticisms. Again no consideration is given to the importance of the doctor-patient relationship. The investigators conclude that "reactions to pain, fear, and anxiety produced pallor of the mucosa and inhibition of mobility. . . . " Anger, resentment, and hostility generally gave rise to

hyperemia and engorgement, with moderate increase in colonic contractions. . . . When the patient was especially depressed, angry, or resentful the engorgement and hyperemia of the colostomy appeared to be more pronounced." It should be noted that these writers describe different reactions to anxiety and to depression than do Grace et al. and again we are left with no statement of what criteria were used.

Comment: The studies here reviewed, while perhaps indicating trends, mainly illustrate some of the as yet unsolved difficulties of method and reporting in this kind of research. In my opinion none of these studies successfully overcame these difficulties and consequently the published conclusions must be regarded with caution. Neither the design of the research nor the methods used, physiologic or psychologic, provide either the investigator or the reader with the necessary information to test the validity of correlations. An entirely different research design is necessary to answer these questions, one which will permit statistical examination of data. Such a design has been developed and will be published elsewhere.[25a]

Psychophysiologic Correlations during Psychoanalysis. While the psychoanalytic technic has yielded much valuable information on the psychodynamics of these patients, so far there have not been any systematic attempts to correlate the psychologic and somatic processes during psychoanalytic study. What correlations have been indicated still fall in the category of clinical impressions and require much more careful evaluation before their validity can be established.

A CRITIQUE OF PSYCHOLOGIC FORMULATIONS

Data have been presented to show that processes which can be demonstrated and examined by psychologic technics are frequent, perhaps necessary, but not sufficient conditions for the development of ulcerative colitis. We are now in position to summarize these psychologic data and to examine the various psychologic formulations that have been proposed. In brief, these patients tend to fall into a population group having preponderately pregenital character traits, especially compulsive and dependent features; they show a defect in their capacity to relate to people, with a tendency to retain features of their early mother-child symbiotic relation; there is a failure to resolve this symbiotic relationship, which genetically and dynamically also appears to be related to the distinctive characteristics of one parent, usually mother, who also cannot relinquish this relationship with her child; sexual maturity is not achieved and the patients tend to make marriages which assure continuation of the same kind of relationship; ulcerative colitis develops in settings in which the important relationship in fact or in fantasy is threatened or actually disrupted, when at the same time the patient feels helpless to cope with the new situation; remissions occur when an effective relationship is again achieved; in similar situations, when more effective adjustive mechanisms are operative and when the affect is other than that designated by such terms as helpless, hopeless, despairing, etc., other symptoms, notably headache, rather than colitis develop; there is a vulnerability to psychotic reactions greater than the general population.

These are oversimplified topical statements, the details of which have been developed earlier in this paper, but they serve to focus attention on the psychologic processes which seem important in ulcerative colitis. When the various psychologic formulations are summarized, it will be noted that in general they are in good agreement but that different investigators have tended to emphasize different aspects of the same material. An attempt will be made to reconcile these differences.

1. Alexander.[1] This writer emphasizes the importance of emotional factors which from early life are associated with excremental and alimentary functions. In the precipitation of the disease and of relapses he emphasizes the frustrated tendency to carry out an obligation, be it biologic, moral or material, and the frustrated ambition to accomplish something which requires the concentrated expenditure of energy. This formulation is largely in terms of the psychology of the anal period of psychosexual development. The fact that colitis frequently appears when the patient is facing a life situation which requires some outstanding accomplishment for which the patient feels unprepared is formulated in terms of a regression to that phase of development in which the infant experiences the excremental act as the giving up of a cherished possession on one hand and an accomplishment on the other. In other words, a regression to the anal form of giving or accomplishment may take place. He emphasizes that anal regression of this type is

common in all kinds of diarrhea and in psychoneurotics who do not display any somatic symptoms.

The specific dynamic pattern in diarrhea is formulated as follows: "Frustration of oral dependent longings → oral-aggressive responses → guilt → anxiety → over-compensation for oral aggression by the urge to give (restitution) and to accomplish → inhibition and failure of the effort to give and accomplish → diarrhea."

Comment: In the previous review we presented the evidence that somatic processes having to do with diarrhea are not primary in ulcerative colitis.[19,20] There is therefore question as to whether the formulation related to diarrhea necessarily applies in ulcerative colitis. Nonetheless the facts are indisputable that the anal regressive material described by Alexander is clearly demonstrable in all patients with ulcerative colitis. This apparent inconsistency can be reconciled if we do not require in our formulation that the anal regressive processes have a causative relationship to the primary processes in the bowel. Actually there is no evidence to link the mucosal-submucosal reactions of ulcerative colitis to the psychophysiologic relationships of the toilet training period of childhood. But even without hypothesizing such a relationship, it will be recognized that the conditions under which the disease develops as described by Alexander are also those having to do with the threat to a key relationship. His formulation correctly focuses attention on some of the distinctive aspects of character formation in these patients, but not adequately defined is the matrix in which this personality formation takes place, namely the special characteristics of this particular mother-child symbiosis. It remains to be seen whether and to what degree the prominence of so-called anal traits is contributed to by the developmental aspects of the mother-child relationship and/or by some biologic features of the bowel leading to an early and abnormal emotional investment in excretory function. Seen in this light we consider the unsuccessful attempts to perform as part of the attempt to satisfy the requirements of the key relationship, and the failure to perform as an expression of the helplessness which ensues when the relationship is seriously threatened or cannot be maintained. On the other hand, the possibility remains that anal-regressive emotional stimuli may have something to do with the fact that this particular reaction occurs in the colon, rather than elsewhere, and the probability that once the pathologic process in the colon is operative such stimuli will secondarily influence the function of the bowel. However, there is no evidence that the primary process in the bowel wall has any such substitutive or symbolic meaning. Indeed, there is no indication as to what adaptive function, if any, the primary process in the colon serves.

2. Szasz.[72,73] Essentially similar criticisms of Alexander's formulation of diarrhea, which most certainly apply with greater strength to ulcerative colitis, have been made by Szasz even though he also assumes diarrhea to be the primary process in ulcerative colitis. Szasz's suggestion is that colonic activation and inhibition, leading to diarrhea and constipation, represent the physiologic sequelae of certain alterations in the upper gastroenteric tract, of which the gastrocolic reflex represents the prototype. A critical consideration of this idea from the physiologic point of view, as it relates to the pathogenesis of ulcerative colitis, has been discussed elsewhere.[21] From the psychologic point of view, according to Szasz, mobilization of strong (unconscious) oral-incorporative strivings would be accompanied by constipation while a sudden decrease in such strivings, usually because of guilt feelings, would result in diarrhea. Accordingly, the bowel symptoms do not express any primary psychologic meaning but are interpreted as manifestations of a vegetative neurosis, i.e., as the remote physiologic sequelae of oral tensions.

Comment: This concept did not specifically develop out of a study of ulcerative colitis and is not proposed by Szasz as a formulation for ulcerative colitis. It is important, however, in that it emphasizes the possible role of oral rather than, or in addition to, anal tendencies in this disease. Szasz derived his concept from psychologic data the validity of which is not dependent on the correctness of the gastrocolic reflex concept or its application to the pathogenesis of ulcerative colitis. From the psychodynamic point of view the role of oral (assimilative) processes in the development of the mother-child relationship is well established, and hence Szasz's formula can also be related to how the person deals with the key relationship. Intensification of strong oral incorporative strivings could be expressive of an active attempt to hold on to or restore the relationship, while their disappearance could indicate a weakening or giving up of

the tie. Passive helplessness might be associated with the latter.

3. Sperling.[65–67] This investigator, no doubt because much of her work has been with children, has concentrated on the characteristics of the mother-child relationship. She stresses the contradictory attitude of the mother, who on the one hand unconsciously attempts to maintain the child in a life-long dependence to satisfy her (mother's) own needs, and on the other hand shows strong unconscious destructive impulses toward the child. The latter are particularly likely to become intensified when the child fails to satisfy the mother's needs or when the child's attempts to satisfy these unconscious needs mobilize guilt or anxiety in the mother. The child then becomes the scapegoat for the mother. According to this concept the colitis develops when a change occurs in this specific relationship with mother (or her substitute). Once this relationship is discontinued it will depend upon the patient's willingness and ability to find a replacement for the lost or given-up love object. When the patient is unable to accomplish this, intense frustration ensues, there is an acute increase in destructive impulses, which, however, remain repressed and are discharged through the symptom of bleeding. "The destruction and elimination of the object through the mucosa of the colon (bleeding) would seem to be the specific mechanisms in ulcerative colitis." The feces and blood represent the devaluated and dangerous objects. Sperling believes that ulcerative colitis shows great resemblance in behavior, dynamics and personality structure to melancholia.[66] She emphasizes the degree of sadism turned inward. "The choice of the organ is determined by oral and anal fixations, the colon being the eliminatory organ. The anorexia, vomiting, abdominal pain, diarrhea and bleeding represent expressions of and defenses against aggressive incorporation of the frustrating object."

Comment: Sperling's emphasis on the developmental aspects of the earliest mother-child relationship is one with which this writer finds himself in strong sympathy. Yet much work remains to clarify the details of this relationship and especially whether there are elements specific for the development of ulcerative colitis. Relatively under-emphasized in this formulation is the child's contribution to the difficulties of the relationship. To what degree and in what ways does the original endowment, the biologic constitution, of the child contribute to the peculiarities of this particular mother-child relation?

Whether the mucosal reaction is a pregenital conversion symptom, as suggested by Sperling, is a moot point. As pointed out by Alexander[1] psychologic data alone will not distinguish between secondary symbolic elaborations of bodily processes (e.g. bleeding and diarrhea) and primary psychogenic factors. While the patient may symbolically elaborate the bleeding and diarrhea as "the destruction and elimination of the object," it is not justified to conclude from such psychologic data that the breakdown of the mucosal surface is a direct consequence of destructive impulses turned inward toward the introjected object. This is a psychologic concept, derived from psychologic data, and it remains to be seen whether it can have this kind of pathophysiologic consequence. Present methods do not permit an answer one way or the other, although such an idea gains credence from Seitz's success in provoking exudative skin lesions by hypnotic technics.[63]

4. Lindemann.[41,42] The significance of the loss of a key person and the prominence of morbid grief as a psychic manifestation has also impressed Lindemann. He too points out some of the similarities to melancholia. He offers no hypothesis as to how these processes bear on the development of the somatic pathology.

5. Prugh.[57–59] Working with children, Prugh stresses the reservoir of repressed anger, developing in settings of loss of security, and points out the inverse relationship between symptoms and the expression or acting out in play of aggressive and hostile emotions. He implies that the bowel disorder is in some way related to the inability successfully to discharge aggression, a vegetative neurotic symptom as defined by Alexander.[1]

6. Karush and Daniels.[38] On the basis of the psychoanalysis of two women patients, the initiating and perpetuating psychodynamic factors for these two patients are summarized as follows: "The patients never succeeded in building psychological stability, which normally cushions the blow of a serious loss or frustration. They elaborated a precariously effective adaptive system and, in the face of an emotional blow, the apparatus for effective action required to cope with the emergency was so disturbed that profound anxiety and rage, long suppressed, now threatened to overwhelm them. With such a loss of successful mastery, combined with character-

istic over-reaching ambition, the most common response was *depression.** Instead of acting out hostility toward denying or frustrating figures they were then overcome by a sense of *futility and hopelessness** and retreated further to infantile clinging and dependency." The authors point out the peculiarly intense effect on their psychologic adaptation of the subjective perception of the severe physiologic derangements, with secondary, largely unconscious, elaboration in archaic, symbolic terms. They suggest that the ease with which this type of regressive behavior occurs may be proportional to the intensity of "a constitutional, schizoid, emotional, integrative defect."

They emphasize that the physiologic changes in the colon cannot be discussed in purely psychologic terms and that the psychoanalytic investigation of adults cannot resolve the enigma of "organ selection." They raise the possibility that the same kind of chemical and neurophysiologic defects that interfere with integrative activity of the central nervous system, reflected in disintegrative psychologic responses, also produce the disintegrative behavior on the colon.

Comment: This formulation stresses the crucial importance of depression in ulcerative colitis. It is related by the authors to the dependency in childhood. While the problem of object relations is not specifically discussed, it is clear that the conditions necessary to establish and maintain relationships are important factors in the vulnerability to depression in these patients. In contrast to some of the other formulations they do not attempt to relate directly the aggressive or destructive impulses and the bowel changes, either in symbolic terms or as vegetative discharge, although their formulation would not exclude either possibility.

7. *Groen.*[35] This author sees ulcerative colitis developing when persons with certain character traits are in conflict situations which involve an acute love loss. He stresses the humiliation suffered by the patient as well as the loss of sources of support and the fact that they are unable to cope with the situation. "The repression of this special kind of defeat, the outwardly 'normal' behavior, the finding of a pseudo solution, while the insufficiency of their own personality has been so rudely revealed to them, the carrying on while they were standing in life helpless, loveless, and without confidence in their own personality—all this appeared to belong to the typical reaction of these personalities."

Comment: While this is a good description of some of the manifest behavioral processes in the patient with ulcerative colitis, it contributes little to the understanding of how this development comes about.

8. *Mushatt.*[52] This author suggests that a basic problem is that the relationship to the outside world is made on an oral incorporative level. "This relatedness is expressed through bodily imagery by means of the gastro-intestinal tract, and from this concept the disorganized gastro-intestinal function can be considered both as a preverbal or visceral communication of primitive psychic activity and as a defense against psychic disorganization." He also sees a similarity to melancholia. Prominent in this concept are the distinctive aspects of the oral level of development, namely a kind of fusion in which the individual feels himself a part of the person on whom he is dependent, the dependency characteristic of the very young and helpless child on its powerful parent, and the attitude that the object is an inexhaustible source of gratification. He points out the ambivalent character of the identifications and the tendency to internalize conflicts in relation to the outside world and to express them through the gastrointestinal tract. The individual's difficulties seem to arise from defects in ego development which impair his capacity to relate to people and to master the environment. These defects are made possible by prolonged exposure to an unfavorable emotional climate, initially in relation with the mother, later with the father. The mothers are often unconsciously rejecting or using the child for their own end. "As a result of these circumstances the individual seems basically to be in a state of depression and secondary chronic rage held in abeyance by a suitable dependency relationship. When such a relationship is disrupted the individual feels helpless against the world, depressed and filled with primitive rage. This rage, in turn, arouses intense fear of loss of love and fear of destruction at the hands of the environment. Internally this finds expression in loss of self-esteem, intense guilt, and fear of punishment from an archaic superego." The author attempts no explanation as to how such processes eventuate in the colitis.

Comment: Mushatt's formulation is valuable in that it relates developmentally the symbiotic

* Reviewer's italics.

mother-child relation and the oral-incorporative (assimilative) process whereby this comes about. He is the only writer who has emphasized the defect in ego development implicit in the difficulties in relating to people. Like previous formulations, the significance of loss of object relationship and depression and the similarity to melancholia are brought out.

9. Grace, Wolf and Wolff.[32] These investigators have provided a description of some aspects of the manifest personality and behavior and of the settings in which the disease develops. Other than some very general concepts of "stress" mobilizing an ejection-riddance pattern involving the large bowel, related to feelings of "hostility," "anger," "resentment," "anxiety" or "apprehension," they propose no specific psychologic formulation.

ATTEMPT AT PSYCHOSOMATIC FORMULATION

I make no pretense at being any more successful than previous investigators in achieving a formulation which satisfies all the known facts. Actually there are too many gaps in our knowledge to permit success at this time. My effort, therefore, will be directed as much to indicating the requirements for a formulation, the areas in which further definition is necessary, as it will be to the formulation itself.

In the first place we must identify the structural and functional locus of the disease. It does not suffice to say that this is a disorder of the bowel. It is a disorder which involves particularly the lining surface of the bowel and very possibly those functional components which are necessary to maintain an effective and selective barrier against penetration into the organism. In addition to mucosa and submucosa, this would probably also include the local circulatory systems as well as elements of the reticuloendothelial system. In this regard it must be remembered that the lumen of the bowel is outside the body and that the lining surface, although of entodermal origin, is, like skin, part of the boundary between the body and an external environment. Further, this "external" environment within the bowel must be considered in ecologic terms, since it includes living organisms existing in a dynamic relationship with the human host. The occurrence of disorders endoscopically indistinguishable from ulcerative colitis in some patients receiving antibiotics brings into consideration the processes concerned with the maintenance of a dynamic steady state between these two environments.[75] While little is known of the dynamics of this process, it may be helpful to include in one's perspective the concept that there are conditions in the host under which the ecologic balance is disturbed or the protective barrier fails. This is not a new idea in ulcerative colitis[29] and has also been suggested in amebic colitis.[12] For convenience I shall refer to these as the conditions bearing on host resistance, where I use this term in the broad sense of all processes having to do with the maintenance and integrity of this membrane in relationship to its environment. Needless to say, this might include processes which have psychologic or behavioral aspects.

Having first posed the problem in terms of a limiting membrane between two environments and the tissue components involved, it is also necessary to consider organ function. Here we deal with the eliminative functions, which are largely motor, aided by some secretory activity.*

Embryologically, the lining membrane develops before the muscular coat.[64] The primitive gut begins with the establishment of the entodermal layer within the spheroidal blastocyst. When the mesoderm forms, its splanchnic layer becomes associated with the entoderm. The entoderm gives rise to the epithelial lining while the mesoderm supplies the muscular and connective-tissue layers of the gut wall. The primitive hind gut ends blindly and it is only at about the sixth week that a connection with the outside is established with the proctodeum. Meconium begins to accumulate in the fourth month but coordinated motor function probably does not develop until some time later. Defecation *in amnio* rarely occurs in the human fetus.[79] The eliminative activity becomes coordinated under the control of the vegetative nervous system and

* The resorption of water, leading to formed feces, may be thought of as a special aspect of the eliminative function, one which involves the absorptive functions of the lining membrane. This function develops later than the purely eliminative function. The infant's stool is initially relatively fluid and only over a period of weeks or months finally achieves the solid consistency of the adult's.[30] This is not simply a consequence of the infant's liquid diet but involves a developmental process as well, as is indicated by the fact that liquid diets at later ages do not result in diarrhea. Further, experiences with ileostomy and colostomy prove that this function can be taken over by terminal ileum or proximal colon even at an advanced age. Motor function is involved, both in respect to infant colon and adult terminal ileum or proximal colon, since formed stools do not develop until motility has slowed.

later in childhood comes partially under voluntary control through mastery over striated muscle, including the sphincters, a developmental and learning process. One might thus construct a developmental and functional hierarchy, ranging from the earliest boundary, and later barrier, function to the highly integrated, learned bowel control behavior; from cellular-tissue function to organ function to total human behavior, which has psychosocial components. It is my thesis that different kinds of behavioral and psychologic phenomena are associated with these different levels of organization. The problem now is to see what clarification of these differences is possible in ulcerative colitis, since more than one process may be operative simultaneously in the same person.

While evidence of disturbed bowel function and disturbed behavior around the act of defecation often precedes the onset of ulcerative colitis, it is the tissue change which marks the transition to ulcerative colitis. The major psychologic phenomena which have been found to be associated with this transition are (1) some disturbance in a key relationship, real, threatened or phantasied, and (2) an affective state characterized by such terms as helplessness or despair. I suggest that the helplessness consequent to the disturbance in the key object relationship may be regarded as evidence of a traumatic separation process and that such a state may be accompanied by biochemical or physiologic derangements which permit initiation of a variety of pathologic processes in tissues including those characteristic of ulcerative colitis. This general hypothesis is based on the clinical observation over many years that a wide variety of major derangements in tissue function and structure seem to occur in settings of disrupted human relationships.[24] From the beginning of life, survival depends on the maintenance and development of the relationship of the child with some adult, generally mother. Ego development can only take place through object relationships. There is a growing body of knowledge concerning the effects of loss of or absence of object on child development. The observations of Spitz indicate that these may include major physiologic derangements.[67a] Loss of object at any time of life poses a major adaptive task. Although not yet studied scientifically, it is folklore knowledge as well as the experience of the perceptive physician that people may fall ill and die after the death of or separation from important family members. Grief, the normal response to a loss, is a process having both psychologic and physiologic aspects. Freud has pointed out the reparative and reconstructive aspects of grief as a means of overcoming a loss.[26] As many observers have noted, the patient with ulcerative colitis is often one who has failed to accomplish the work of grief, who has achieved no successful psychologic adjustment to the loss. It is suggested that this provokes a complex psychophysiologic state, a psychologic representation of which is the affect, and a somatic aspect of which is some process or processes eventuating in the tissue derangement.

The phenomena just described would be very general ones and might also, in other individuals, lead to other kinds of tissue derangements. We have observed similar settings for some cases of arthritis, asthma, dermatitis, malignant phase of hypertension, carcinoma, leukemia, Hodgkin's disease, congestive heart failure, to mention only a few.[24,32a] Why is the colon the affected site in these patients? (Actually, of course, the colon is not the only site.) Is there evidence for any kind of primary defect, in the colon, developmental or acquired in foetal life or early infancy? Evidence on this point is very sparse. The rare occurrence of ulcerative colitis in the newborn and very young infant speaks in favor of such an idea.[4] The incompleteness of the information on bowel function in the infancy of these patients has been noted but it is fairly well established that some kind of bowel dysfunction, usually constipation, long antedates the colitis. This, however, cannot be taken as evidence one way or the other for some primary defect in bowel wall and hence this remains an important subject for further research. If such defects exist, developmental or acquired early in infancy, it may be assumed that they contribute to the localization of the disorder in the bowel as well as to total personality development. The biologic characteristics of the body and its parts must be involved in the adaptive and developmental maturation of the individual and therefore also in the psychologic expressions thereof. In such an instance we would assume that the bowel would become specially invested emotionally and would more likely become an organ through which both pleasure and aggression might be expressed. Further, these processes would be implicated in the transaction between mother and child.

Since we cannot establish at this time whether

or not the bowel of the future ulcerative colitis patient is biologically distinctive, so to speak, I must leave this as an open question, which may be included or not in the formulation. What seems less uncertain is that the mother-child symbiosis is distinctive, and herein may lie factors which will determine the specific psychologic vulnerability of the individual and may contribute to the predilection for bowel trouble and headache as well. Present knowledge does not permit us to spell out details and, indeed, I believe the microscopic scrutiny of this relationship is the next required step in the psychologic elucidation of ulcerative colitis. It is also difficult to write about, because "mother-child symbiosis" is a transactional concept; it concerns the transactions whereby mother and child mutually relate and affect each other and it becomes misleading to try to describe mother and then child or vice versa.* The mother attempts to gratify certain needs through her child and may be frustrated and react in some way when the child fails to fulfil the need. The child, on the other hand, has certain basic needs, indeed needs which go to the very root of survival, which can only be achieved in the relation with mother or mother substitute. By virtue of the mother's behavior and character structure and/or by virtue of the inborn endowment of the child, its capacity for adjustment, these children grow up with serious defects in ego function. Benedek has pointed out how the child, in order to reduce the danger of separations, incorporates mother's conflicts, thereby adapting to mother's conflictful behavior.[3a] They may then remain permanently dependent on mother or on a limited number of substitutes, without whom they are literally helpless, since they cannot function in certain ways, having never successfully achieved independent means to do so. Elsewhere I have introduced the concept of "surrogate ego" functions to refer to these processes.[25b] Herein lies the overwhelming trauma of separation and the "curative" effect of a certain kind of doctor-patient relationship. I would list some of the important qualities of mother and of child which are implicated in this development as follows:† (1) The mother has an unresolved involvement with her own mother, which is often transferred to this one child. The details of this require further examination. (2) For a variety of reasons the mother's relation to the child is a "conditional" one, meaning that it may be relatively warm and succoring only when the child's behavior does not mobilize anxiety or guilt in mother. Transactionally, some of the child's intrinsic behavioral patterns, such

* Transaction is defined in Webster's New International Dictionary (1935) as, "An action or activity involving two parties or two things mutually affecting or reciprocally influencing one another." Dewey and Bentley[17a] have pointed out that man's organization and presentation of inquiry into natural processes has shown three successive levels of development. The first views of nature were *selfactional*, where things were viewed as acting under their own power. This corresponds to the magical, religious, animistic, or vitalistic concepts of the past centuries. Then with Galileo, Descartes and Newton there came into use *interactional* concepts, in which thing is balanced against thing in causal interconnection. This corresponds to the mechanistic, linear, cause-and-effect, stimulus-response concepts that have furnished the dominant pattern of scientific procedure, especially in medicine, up to the present. This is still a useful approach, under certain well defined conditions, but has imposed serious limitations on our understanding of natural processes. The transactional approach is ushered in by Einstein's field theory, which brought time and space into consideration and prepared the scene for Newton's unalterable particles to go the way of time and space. In the *transactional* concept, one observes processes, the "thing" in action, where the "thing" and the "process" are not separable one from the other. Thus in the case of organism and environment, *interaction* assumes organism and its environmental objects to be present as substantially separate existences or forms of existence, prior to their entry into joint investigation. *Transaction*, on the other hand, requires the acceptance of organisms and environment as a common system, and assumes that no preknowledge of either one alone as adequate; the organism is observed in process with environment. These concepts are now being developed in terms of open systems, dynamic steady states, feed-back mechanisms, and multifactorial concepts. It replaces the linear with the spiral, meaning that no system is ever again exactly the same as it was before. The patient is never "restored" to the same state of health that existed before he became ill; it is a different state. Every human relationship changes all participants. Health and disease must be understood in transactional terms.[23]

† Again I must emphasize the transactional aspects, the mutual feedback, as well as call attention to quantitative and qualitative differences. Some children are more vulnerable, some mothers more "pathogenic"; some children are more "pathogenic" to their mothers, with disastrous feedback; some families find themselves, through circumstances outside their control, in life situations which accentuate these problems. Otherwise we would be unable to account for the first attack of colitis occurring in childhood as well as in old age; colitis occurring in the person who barely gets by and the person who made a passably good adjustment before falling ill; the occurrence of colitis in one child but not in the siblings. A variety of factors, biologic, psychologic and social must come together before the colitis begins.

as feeding, bowel activity, motor activity, over which the baby has limited or no control, may be among the behavioral processes which evoke maternal anxiety, guilt or shame. Since these are operative from birth on, such tensions between mother and infant could contribute to the oral fixations so prominent in these patients. It is in this area particularly where biologically determined needs, as may be associated with some bowel defect, may contribute significantly to the symbiotic relation. (3) These mothers require a striking measure of control over the motility of their children or the children require control and succeed in enlisting it. This is expressed in the control over intake, motor activity, bowel movements and emotional expression which so often characterizes mother and child. Here again one sees mutual provocations, gratifications and resultant guilt. At this level of object relationship and ego development, fixation at and/or regression to the anal level of psychosexual functioning is mutually stimulated. (4) These children may have a defect in their autonomous capacity to relate to objects. More information is necessary on the development of very early relationships with people other than the mother. These children may also have a defective capacity to develop an independent ego, contributing to the need to continue to utilize others as "external" egos. In this and the preceding point I infer the possible existence of biologic defects as well as specific psychologic stresses from the environment. If such defects exist, however, they differ in degree, if not in kind, from the defects which lead to childhood psychoses.[59a] For the most part the reality testing function of these patients is not so seriously impaired. It is suggested that the difference lies in the fact that the colitis patient was better able to establish and maintain a symbiotic relationship than the psychotic child, but that this particular relationship forever largely determined and circumscribed to a high degree the nature of all other relationships. In terms of ego development this would not lead to as great damage to reality testing functions as occurs when no object relationship is established in the first year or so of life. But it would lead the individual extremely vulnerable to separation, as indeed these people are. (5) The psychosexual development of these individuals is arrested by virtue of the persistence of the symbiotic relationship, the need for "external" ego support. I have already mentioned some factors contributing to oral and anal fixations. A biologic defect ("organ weakness") may also contribute to these limitations. In any event, by virtue of the indispensibility of the key object (usually mother) as external ego, the oedipus complex cannot be resolved and mature sexuality is not achieved.

In this formulation, then, ulcerative colitis develops in particular individuals under specific circumstances. The tissue reaction, which forms the basis of the colitis, develops only when other adaptive processes fail, and the significant object relationship is threatened or actually interrupted. The specific factors that go into the development of this relationship will help to determine the kinds of circumstances and the settings in which this takes place. Biologic and psychologic developmental factors will help to determine the bowel as the site of the tissue breakdown. As long as an object relationship is maintained, other pathologic processes may become manifest, but not colitis. Thus the pathologic character traits constitute means for maintaining the key relationship and satisfying needs within that framework. Other neurotic, psychotic or psychosomatic symptoms (e.g. headache) develop during conflict situations within the framework of this relationship, where the relationship is not seriously threatened. Headaches, for example, develop when the patient takes a stand, expresses aggression, but maintains control. Conscious or unconscious guilt is associated with the headache, but not helplessness.[21] Elucidation of the specific aspects of the object relationship constitutes a most important problem for further research.

In respect to the pathogenesis of the tissue process itself, I can only speculate. I have already indicated my belief that this is a non-specific process, associated with the separation reaction and having no primary psychologic meaning. I suggest that when separation is not or cannot be dealt with by psychologic mechanisms (as in normal grief as well as in some types of neurotic or psychotic processes) physiologic changes are initiated in the body which interfere with or disrupt other adaptive or integrative processes, permitting various types of tissue breakdown, disturbances in local tissue growth, invasion by viruses or bacteria, etc. The nature of these processes remains unknown. Reichsman and I recently have observed in an infant with a gastric fistula a profound alteration in responsivity to histamine during object loss.[25a] This may

be indicative of some biochemical change characteristic of this state.* One possibility in ulcerative colitis is that these as yet unidentified changes may alter relationships in the colon so that it responds to its own flora as to pathogens. Another possibility is that the vascular system is particularly affected by these processes, perhaps becoming reactive to chemical substances elaborated as distant sites, in the tissues, or products of bacterial activity.

Such a formulation does not necessarily exclude the contribution of other mechanisms that have been proposed in other formulations. Thus the gastrocolic reflex[72,73] or the activation of eliminative patterns[1] would both be consistent with the psychodynamics of these patients and might add to the strain on colon. The failure of psychosexual maturation in these individuals might well involve erotization of the colonic mucosa, meaning that the colon becomes the site of physiologic changes, tumescent in character.[25,45,46,64] Beginning with mouth and with sucking, engorgement of mucous membrane and pleasurable sensations are both in the service of pleasure and of object relations. Such a regressive perversion of colonic function might be activated in situations when more advanced levels of object relation are threatened. This again might contribute to the final bowel change. And finally with motility in relationship to external world and objects markedly reduced, with the profound regression that accompanies this state, the turning inward of aggression may be associated with internal bodily changes, including changes in the colon. All of these are psychologic processes which may be expected to follow upon the traumatic separation, but whether they have physiologic aspects which specifically contribute to the colitis cannot be established by psychologic means alone. But we must avoid the error of assuming that the discovery of evidence favoring one mechanism necessarily means that other mechanisms are not also operative and perhaps contributing to the full development of the pathologic process. Indeed, there is every reason to believe that a chain of events results in the final pathologic derangement.

* That we should have encountered some process involving histamine is especially interesting since Feldberg has raised the question whether histamine release represents the first reaction of the mucosa to any form of injury. Also, the intestinal bacteria form histamine from histidine, and many food substances contain histidine.[25c]

SUMMARY

1. The psychologic data in thirty-nine patients with ulcerative colitis have been summarized and published reports on more than 700 patients have been reviewed.

2. Among these patients there was found with impressive consistency (*a*) defects in personality structure long antedating the onset of colitis, (*b*) a characteristic type of dependent and restricted relationship with people, (*c*) consistent psychopathology in the mothers, and (*d*) failure to achieve full heterosexual development.

3. The onset and relapses of the disease were found to occur in settings which represented to the patient real, threatened or fantasied interruptions of key relationships, but the disease developed only when the corresponding affect was helplessness, despair, hopelessness, etc.

4. In conflict situations which were not responded to with such effect, other neurotic, psychotic or psychosomatic (notably headache) reactions were observed.

5. Various psychosomatic hypotheses are critically reviewed.

6. A modified formulation is offered which (*a*) stresses the significance of the mother-child symbiosis in determining the particular personality development and vulnerability to separation, (*b*) relates the tissue reaction of ulcerative colitis to biologic changes occurring consequent to traumatic separations, (*c*) and attempts to account for the choice of organ (colon) on the basis of constitutional and experiential factors in the mother-child transaction.

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Seminars on Carbohydrate Metabolism

Current Views on the Mechanisms of Insulin Action*

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THIS paper will try to develop a picture of the current concepts of the problem of the action of insulin upon intermediary metabolism. The theoretic background necessary for a reasonable understanding of the problem will be discussed. Then the experimental evidence which at this time seems most significant will be presented and evaluated. Necessarily, the great mass of literature at hand makes selection imperative, and much data will be omitted which some may think of prime importance.

The problem of the action of insulin may be said to have begun with the demonstration of Minkowski and Von Mehring that a state closely resembling diabetes mellitus could be produced in the dog by surgical removal of the pancreas. These experiments demonstrated beyond doubt that the pattern of defective metabolism associated with diabetes was due to the absence of some humoral principle elaborated by the pancreas. The discovery of insulin by Banting and Best followed in 1922. This initial phase in the history of the problem of insulin action did not end here but rather with the demonstration by Houssay and Biasotte (1931)[19] that experimental diabetes could be ameliorated by the simultaneous removal of the anterior pituitary. The demonstration by Long and Lukens (1936)[28] of similar amelioration by simultaneous adrenalectomy completed the picture: The metabolic patterns which result from the activity of tissue enzyme systems are under the control of the complex interplay of a series of hormones whose balance or imbalance produces normal and beneficial homeostasis or profound and often fatal aberrations.

The picture which emerged from these early studies is greatly oversimplified and is a familiar one. There is in diabetes an accumulation of glucose in the body giving hyperglycemia and glycosuria. A decreased storage ability results in lowered glycogen content of muscle and liver. The low respiratory quotient is attributed to decreased or absent oxidation of carbohydrate. The disturbed fat metabolism is manifested by marked loss of body fat and fatal ketosis. An increase of protein catabolism results in a loss of body tissue and increased nitrogen output.

In this early period of study on the problem of the action of insulin experiments on the whole were limited to studies of total metabolism involving measurements of blood sugar, shifts of carbohydrate stores from one organ to another, respiratory quotients, change of total protein metabolism, effect of altered diabetic state upon ketosis, and the like. Experiments were mostly confined to the intact animal and were concerned with measurements of glycogen, lactic acid, ketones, and so on. The ratio of dextrose to nitrogen in the urine (D:N), for example, was a favored method for evaluation of the severity of the diabetes. It is interesting to note that Lusk in his Harvey Lecture in 1904 on "Metabolism in Diabetes Mellitus" makes no reference whatever to "intermediary metabolism."

This period was dominated by what might be called the monistic view: namely, that the metabolic defect associated with diabetes was due to the lack of one hormone. Further, in the total diabetic there was 100 per cent disability. Two main theories were proposed to explain this. Reduced to its elements, these views are expressed with great brevity in Table I. Underutilization meant that there was complete inability of the organism to oxidize carbo-

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hydrate. The metabolic requirements were met entirely by fat and by that part of protein which was not glucogenic. The respiratory quotient, of 0.7, was that of the burning of pure fat. In the fasted diabetic, (D:N) expressed the ratio of the dextrose to nitrogen found in the urine. In

TABLE I
UNDERUTILIZATION VERSUS OVERPRODUCTION

Respiratory quotient	0.7
D:N ratio	3.5
Fat to carbohydrate	C_2 to C_4 (?)

the fed animal this ratio was corrected for the ingested food, including that portion of the protein and fat which was assumed to be convertible to carbohydrate. The value commonly used is 3.0 to 3.5, based upon limited experiments with dogs or a few human cases assumed to be totally diabetic. This value was explainable on the assumption that about 50 to 60 per cent of the amino acids of protein were converted to glucose and were excreted unused. Fat was never a source of carbohydrate.

The opposing view held that no impairment in the ability of the organism to oxidize carbohydrate resulted. Hyperglycemia was regarded as a compensatory mechanism which enabled the diabetic to oxidize carbohydrate. But overcompensation resulted in glycosuria. The extra glucose necessary for this hyperglycemia was derived not only from protein but from fatty acids. (The glycerol portion of the fat was always calculated with the carbohydrate.) The precise reactions by which fatty acids were converted into carbohydrate were never clearly explained.

Rumblings of this controversy still continue but the introduction of tracers in metabolic studies has settled it. The kernel of the situation is expressed in the table by C_2 to C_4(?). It has been abundantly shown that all fat metabolism goes through the formation of the two-carbon compounds—acetyl-coenzyme A. This condenses with four-carbon compounds (oxalacetic acid) to enter the Krebs cycle for further conversion or oxidation. Any member of the Krebs cycle may be incorporated into protein, fat or carbohydrate. Hence it is possible, as has been shown experimentally by tracer studies, that carbon atoms derived originally from fatty acids may be found in carbohydrates. But the important distinction is that the four-carbon compounds necessary for combination with the two-carbon compounds always come from preexisting carbohydrates and never from fat. Hence a net new formation of carbohydrate from fatty acids does not occur.

We can say the same thing in another way: An obligatory step in the formation of carbohydrate from fat would be the condensation of two C_2-compounds (monocarboxylic acid) to a C_4-compound (dicarboxylic acid). But this so-called Thunberg reaction has never been demonstrated to occur in mammalian tissue.

The demonstration by Houssay and Biasotte (1931)[19] and by Long and Lukens (1936)[28] that the diabetic state could be ameliorated by concomitant removal of the pituitary or adrenals initiated a new phase in the problem of the action of insulin. Carbohydrate metabolism and concomitant fat and protein metabolism were obviously under hormonal control involving at least three enzyme systems. Furthermore, the monistic theories suggesting that the diabetic organism has only one metabolic defect, either an inability to oxidize glucose or a gross overproduction of glucose from fat or protein, became untenable. It was obvious that all three types of metabolism were under multiple hormonal control.

Simultaneous with these interesting developments in the field of diabetes a new field began to dominate thought and experiments in metabolic studies. This was the spectacular development of enzyme chemistry in the first half of the century. Buchner first demonstrated the catalytic activity of an enzyme system dissociated from the living cell. There followed from laboratories all over the world demonstrations of the properties of enzyme systems derived from living tissues. Many of these enzyme systems were isolated in pure form and were shown to catalyze, with high specificity, single chemical reactions. Many were crystallized and the cofactors necessary for their action were isolated and identified. The innumerable chemical reactions involved in the total metabolism of food stuffs were demonstrated and, although knowledge was still incomplete, patterns of chemical reactions were mapped which described these metabolic processes in detail. Thus the study of intermediary metabolism, as distinguished from total metabolism, became dominant. The experimental use of surviving tissues, such as heart-lung preparations, eviscerated animals, perfused isolated organs, surviving tissue slices or homogenates, and tissue extracts became widely used as new experimental tools.

This new approach rapidly brought insight

into the problem of the action of insulin. For the sake of simplicity we may discuss this in two ways. For want of better terms we may call the first the physiologic approach and the second the molecular consideration. The physiologic approach endeavors to study an isolated chemical reaction occurring in the course of intermediary metabolism of carbohydrates, proteins and fats to determine whether it is inhibited or accelerated by the presence or absence of insulin or insulin antagonists. Thus the "biochemical lesions," as they have come to be known, which occur in the diabetic may be written in the form of chemical reactions as aberrations from normality. Most of the reports in the literature are concerned with this type of study. In opposition to this, the molecular approach has a different aim. For example, we know that enzymes influence reactions of foodstuffs in such a way as to bring about their transformation, final oxidation or synthesis more rapidly than would be the case if the catalytic action of enzyme systems was not available. The molecular mechanisms by which this is brought about are little understood. Molecules are known to exist in different states of reactivity and the presumption is that enzymes bring about a higher concentration of the reactive forms so that the "energy hump" which hinders the desired reaction may be overcome. However, one mechanism has been well established, namely, the combination of substrates with enzymes according to the Michaelis-Menton concept (vide Clark, 1952[8]). Presumably such combination is a prerequisite for enzymatic action. What is the relation of hormones, particularly insulin, to such an enzyme-substrate complex? Little is known of this aspect of hormonal action except perhaps that the binding of insulin to tissue (vide infra) may be a necessary requisite for hormonal action. Possibly hormones change configurational patterns of enzymes in such a way that substrate combination becomes radically altered. The precise mechanism by which hormones interact with the enzymes of the tissue remains to be elucidated and, until this has been done, there can be no fundamental concept of the nature of diseases associated with endocrine imbalance.

These preliminary remarks about the general aspects of intermediary metabolism make a consideration of the possible biochemical defects due to a deficiency of insulin or to an excess of contrainsulin factors more intelligible. The possible sites of insulin action which are most prominent in current discussions of the biochemistry of diabetes are: (1) increase of cell permeability or transfer of glucose across cell barriers to enzymatic sites; (2) acceleration of the hexokinase reaction; (3) increase of high-energy phosphate formation, and (4) effect on oxidative reactions of the Krebs cycle. A consideration of these and a knowledge of the glycolytic cycle and its relation to the Krebs cycle (outlined in the schema shown in Fig. 1) form a framework about which the problem of the action of insulin may be discussed. A preliminary consideration of the nature of these defects will be given first, followed by a more detailed presentation of the experimental evidence upon which the concepts are based.

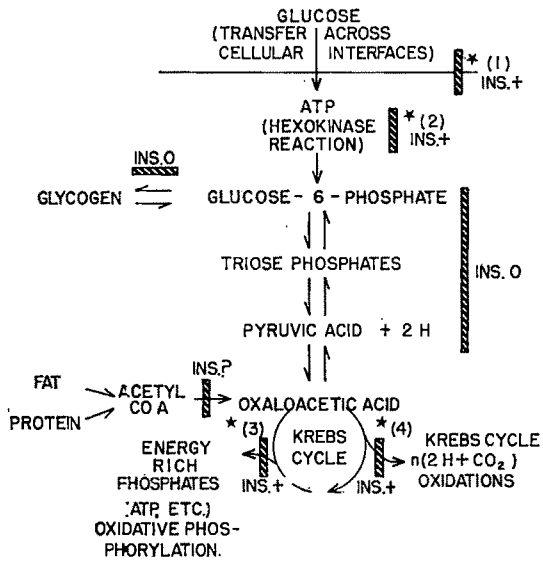


FIG. 1. Current schema of glycolytic and Krebs cycles. Possible "metabolic defects" associated with insulin deficiency are indicated by hatched blocks: (1) glucose transfer, (2) hexokinase reaction, (3) oxidative phosphorylation, and (4) oxidative reactions in Krebs cycle.

The first conceivable biochemical lesion concerns itself with the permeation of glucose through cellular barriers to sites of enzymatic action. The forces which hinder or accelerate the access of glucose to enzyme systems may be of a physical or a chemical character. At present there is no certain way of differentiating the two so that it is best to call the mechanism concerned with the permeation of glucose to enzyme sites a transfer mechanism. It is important to realize that the cell is composed of many subcellular structures such as mitochondria, microsomes, nuclei, and so on. Each of these

cell-particulates is known to contain systems of enzymes concerned with carbohydrate metabolism. In turn, each of these systems is separated by a cellular barrier from its surroundings. These considerations naturally render the problem of the action of insulin upon transfer very complex. We can say no more at present but that there is evidence indicating that the transfer of glucose (and other sugars) to enzyme sites appears to be under hormonal control.

The second possible biochemical lesion in insulin lack is related to the activation of glucose. It is now well established that in practically all mammalian organisms glucose is prepared for further metabolism by the formation of glucose-6-phosphate. Glucose metabolism without phosphorylation, if it occurs at all, is so exceptional that it may be dismissed from further consideration. The formation of glucose-6-phosphate does not occur unless catalyzed by the enzyme, glucohexokinase, which is present in practically all mammalian cells. The reaction (which is essentially irreversible) is:

Glucose + ATP = glucose-6-phosphate + ADP.

The third possible diabetic defect involves the production and storage of high-energy phosphate compounds such as ATP, creatine phosphate or similar compounds. Many reactions depend upon the availability of ATP, for example glucose-6-phosphate formation, synthesis of fatty acids, certain oxidative reactions, synthesis of protein or possibly the penetration of glucose through cellular barriers. It is therefore natural to consider the possibility that defects in the production of high-energy phosphates might be involved in the diabetic state and, indeed, there is much evidence to support this point of view.

The fourth and last diabetic lesion which is considered in the literature is concerned with defects in oxidative reactions, particularly of derivatives of glucose. These oxidative reactions appear to be limited to reactions involving the Krebs cycle. The existence of this type of diabetic defect is not too convincingly supported by evidence in the literature and there is therefore a tendency to neglect it in discussions of insulin action.

Following the initiation of the metabolism of glucose by phosphorylation, glycolysis continues by a conversion of the monophosphate to diphosphate and then by a split into two triose molecules. These 3-carbon compounds form pyruvic acid, the link which joins the glycolytic cycle to the Krebs cycle. The linkage of the glycolytic cycle with the Krebs cycle is by way of a condensation of pyruvic acid with oxalacetic acid. One oxidative step occurs with the elimination of one molecule of CO_2 and the formation of citric acid, the first member of the Krebs dicarboxylic acid cycle. Provision is made in the glycolytic cycle for the storage of carbohydrate. Glucose-6-phosphate is converted into glucose-1-phosphate (by phosphoglucomutase) which in turn is converted to glycogen (catalyzed by phosphorylase). All the reactions of the glycolytic cycle from pyruvic acid to glycogen are reversible. This means that either glucose or pyruvic acid or any of the intermediaries are capable of forming glycogen. The hexokinase reaction is irreversible and glucose can only be regenerated from glucose-6-phosphate by the splitting off of inorganic phosphate catalyzed by phosphatase.

HEXOSE-MONOPHOSPHATE-SHUNT

Much recent evidence in the literature indicates that a pathway of metabolism of glucose, other than through the glycolytic cycle, exists. This involves the oxidation of hexose-monophosphate to phosphohexonic acid. Loss of CO_2 by decarboxylation reduces this compound to a 5-carbon compound which is then split into 2- and 3-carbon compounds. There is no evidence that this so-called hexose-monophosphate-shunt is involved in the insulin problem. Therefore, it will be dismissed from further consideration.

The oxidation occurring in the Krebs cycle are always accompanied by decarboxylations yielding CO_2. In this way the three carbons of the pyruvic acids are oxidized and the original 4-carbon oxalacetic acid is regenerated. The energy yielded by these oxidations forms the high-energy phosphate bonds of ATP which may be retained as such or stored as creatine phosphate. This stored chemical energy is available for a whole series of endergonic reactions (requiring input of energy) such as synthesis of fat, synthesis of protein, acetylation, and so on. It is to be noted that the initial activation of phosphorylation of glucose is one of these reactions. Fat and protein enter into the metabolic schema by the formation of 2-carbon compounds which when combined with coenzyme A enter the Krebs cycle via oxalacetic acid.

Before examining evidence for the hypotheses of the specific action of insulin, some of the recent data bearing on the general question of

the action of insulin upon intermediary metabolism will be discussed. Such a brief review of the recent literature must necessarily be selective and illustrative rather than complete.

With respect to carbohydrate storage as glycogen in liver and muscle, much conflicting evidence is present in the literature as to the role of insulin. Concomitant action of adrenalin and transfer of carbohydrate from liver to muscle complicates these experiments. Bouckaert and deDuve (1947)[2] report experiments which they interpret to mean that insulin is of major significance in the accumulation of glycogen in the liver. They maintain the blood sugar in the experimental animal (dog) at high levels by measured infusion of glucose. The amount of glucose necessary to maintain these levels was assumed to represent glycogen storage. In the absence of insulin only small amounts of glucose were removed from the blood stream but if maximal doses of insulin were given to the preparation glucose storage resulted. The data summarized in Table II show that the eviscerated and hepatectomized preparation retained about the same amount of glucose, but the dog with liver intact required about five times as much injected glucose to maintain constant blood levels. Most of this excess glucose represents extra storage as glycogen in the liver under the influence of insulin.

TABLE II

STORAGE OF GLYCOGEN IN LIVER IN DOG INJECTED WITH INSULIN: STORAGE MEASURED BY AMOUNT OF INJECTED GLUCOSE REQUIRED TO MAINTAIN CONSTANT BLOOD LEVEL[2]

	Gm./Kg./80 Min.	
	Mean	SEM
Control (liver intact)	2.10	± 0.24
Hepatectomy	0.40	± 0.12
Evisceration	0.40	± 0.22
Intact without insulin		± 0

Lang, Goldstein and Levine (1954)[27] objected to this interpretation of Bouckaert and deDuve. They reported experimental observations which they interpret to mean that, following hepatectomy, there is decreased peripheral glucose uptake. This means, they concluded, that there is a humoral factor of hepatic origin which stimulates peripheral glucose utilization. The experiments of Bouckaert and deDuve do not necessarily mean, therefore, that insulin has a direct hepatic action in glycogen storage but might indicate the action of the humoral of the liver on peripheral storage. The difficulty in demonstrating direct insulin effect upon the metabolism of liver has recently been clarified by interesting experiments reported by Renold, Hastings, Nesbett and Ashmore (1955).[33] They report a striking difference between the times of response of the rat diaphragm and liver slices to insulin. In the alloxanized rat the diaphragm responds immediately to the action of injected insulin by increased glucose uptake, glycogen synthesis and CO_2 production. In contrast, hepatic carbohydrate metabolism, as measured by glucose uptake, glycogen synthesis from glucose or pyruvate and CO_2 production, shows only minimal effect of insulin six hours after beginning of administration. Complete restoration to normal values does not occur until twenty-four to forty-eight hours after insulin treatment has begun. In this connection a puzzling difference between the action of insulin upon the carbohydrate and fat metabolism of liver slices in the medium is to be noted. Effects upon the incorporation of labeled acetate into higher fatty acids is easy to demonstrate (Brady and Gurin, 1950;[3] Haugaard and Stadie, 1953[18]) but effects upon carbohydrate metabolism (increased glycogen synthesis, increased CO_2 production) cannot be demonstrated. The recent experiment of Renold et al. (1955)[33] might indicate a possible solution of this difficulty.

The evidence that insulin enhances glycogen storage in muscle is abundant and need not be cited in detail. The diaphragm is used extensively for metabolic studies. The rat is easily altered so that experiments may be performed under a variety of metabolic and endocrine conditions For these reasons rat diaphragm is ideal for *in vitro* studies. Gemmill (1940)[12] first showed that the rat diaphragm survived excellently *in vitro*, utilized glucose from the medium and responded to insulin by utilizing more sugar and synthesizing more glycogen. Mere addition of insulin to the glucose-containing medium increases glycogen storage. In the alloxan-diabetic rat, glycogen storage is sharply decreased and insulin in the medium increases the glycogen synthesis but does not increase it entirely to within normal limits.

Is insulin necessary for glycogen storage? The answer to this was obtained by experiments of Lukens (1934).[29] He examined the breakdown

and resynthesis of muscle glycogen in depancreatized cats at various times. The same level of muscle glycogen was obtained in the depancreatized cat as in the normal cat but the time required to accomplish this was longer.

The effect of insulin upon carbohydrate metabolism in the normal rabbit is well shown by some experiments of Wick et al. (1951)[51] which are summarized in Table III. They used isotopic glucose which was injected into the blood stream of the eviscerated preparation. Subsequently the carcass was analyzed for protein, fat and glycogen for the determination of radioactivity. From these data, as well as from the isotopic carbon recovered in the respiratory CO_2, the glucose equivalents oxidized and the glucose converted to glycogen, protein or fat could be calculated. Compared to the controls, insulin brought about marked increases in all categories: The oxidation of the glucose was increased about fourfold, glycogen storage about fivefold, and incorporation into protein and fatty acids six- and thirteen-fold, respectively. This experiment shows quite well the effect of insulin upon the general metabolic picture.

TABLE III
DETERMINATION OF THE EFFECT OF INSULIN ON GLUCOSE METABOLISM IN EVISCERATED RABBITS USING ISOTOPIC GLUCOSE[51]

	Mean of 4 Mg. Glucose Equivalents	
	Control	Insulin
CO_2	650	2500
Glycogen	130	700
Protein	110	700
Fatty acids	10	130

What about carbohydrate metabolism in the intact diabetic animal? In the early literature, and even up to somewhat recent times, there was considerable confusion in the literature with respect to this situation. Opposing views maintained, on the one extreme, that the total diabetic was totally unable to oxidize glucose. The other extreme view maintained that there was no impairment of glucose oxidation in the diabetic. Some recent experiments by Stetten, Welt, Ingle and Morley (1951)[49] and also by Feller, Chaikoff, Strisower and Searle (1951)[10] using isotopic glucose in alloxanized rats and depancreatized dogs have clarified this situation. The summarized data of Stetten et al. are shown in Table IV.

Normal, alloxan-diabetic and phlorhizinized rats were used in these experiments. These rats under membutal anesthesia were arranged for continuous injection of isotopic glucose, collection of urine and expired CO_2. The injection of C^{14}-labeled glucose was at a constant rate and sufficient to maintain a constant level of glycosuria during the experiment. The time period was up to twelve hours and during the later part of this period not only was the glucose excretion constant in rate but the specific activity of the glucose was also constant. Under these circumstances it was possible to calculate the rate of production of glucose from non-glucose sources. In addition, the determination of radioactivity of expired CO_2 permitted an estimation of the glucose oxidized. The summarized data (Table IV) show: (1) the rate of production of glucose from non-glucose sources and (2) the glucose oxidized to CO_2. The authors conclude "that in the normal rat, the rates of glucose production and oxidation are approximately of the same order. In the phlorhizinized animal, a marked impairment in glucose oxidation is apparent, whereas the rate of glucose formation is unaltered." In the alloxanized diabetic rat "there appears to be a small increase in the rate of glucose production, although statistically this may not be significant. There is little question, however, that a decrease in the rate of oxidation of glucose occurred in this condition, the elevation in glucose concentration accompanying the disease." Feller et al. (1951)[10] reported results on the depancreatized dog which are in substantial agreement with the conclusions of Stetten and his colleagues.

TABLE IV
GLUCOSE UTILIZATION IN ALLOXANIZED RATS[49]

Glucose	Normal (mg./100 gm. rat/hr.)	Phlorhizinized	Alloxanized
Produced	15.0	14.0	21.0
Oxidized to CO_2	11.5	2.6	4.5

The following conclusions may be regarded as being reasonably well established by several groups of workers using various types of experimental animals: (1) Glycogen synthesis from

glucose by liver and muscle persists in the absence of insulin but the rate of formation is diminished. (2) Compared to the normal animal, there appears to be an overproduction of glucose from protein, although the evidence is somewhat conflicting on this point. Net new formation of glucose from fat has not been demonstrated. (3) There can be little doubt that insulin markedly stimulates glycogen synthesis and carbohydrate oxidation in the normal as well as in the diabetic animal. (4) In the alloxanized or depancreatized rat, rabbit and dog, there is significantly diminished oxidation of glucose, but oxidation is not completely abolished.

It is possible that differences within the species may obscure interpretation of experimental results so that judgment must still be reserved. For example, some of the questions have not yet been adequately tested in the depancreatized cat.

EFFECT OF INSULIN ON FAT METABOLISM IN DIABETES

Disturbances of fat metabolism in diabetes have long been known. Early concepts held that the complete diabetic was dependent entirely upon the 2-carbon compounds derived from fat for his energy requirements. The ketones known to be derived from fat were not burned and were excreted as such. This view is no longer tenable.

The concept now accepted is illustrated by the experiments shown in Figure 2. The ability of the liver of the diabetic to synthesize fat is impaired. In addition, large amounts of fat are broken down into ketone bodies by beta oxidation. Certain 2-carbon compounds are formed which, by random redistribution, combine to form 4-carbon ketone bodies. Most of these ketones formed by the liver of the diabetic animal are oxidized completely in the peripheral tissues and are therefore an important source of energy for the animal. A relatively small amount is excreted in the urine. This is well illustrated by the data shown in the illustration. Diabetic cats well treated with insulin no longer produce these excessive amounts of ketone bodies, and slices from the liver of Houssay cats produce essentially none when equilibrated *in vitro*.

The deficiency with respect to fatty acid synthesis, demonstrated by the experiments of Stetten and Klein (1946),[48] are summarized in Table v. These authors determined the rate of synthesis of fatty acids in the normal and in the diabetic rat by means of heavy-water ingestion. From the deuterium incorporated into the fatty acids it is possible to calculate comparative rates of formation of fatty acids from different substrates such as glucose or acetate. The data in

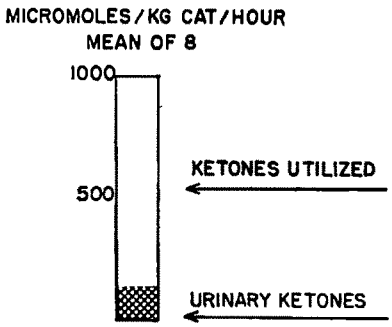


FIG. 2. Uncontrolled fatty acid metabolism of diabetic cat liver (depancreatized cats). Ketone formation by liver slices from fatty acids accounts for almost all of the oxygen uptake; most of the ketones formed are utilized by the muscles; liver slices from normal cats produce little or no ketones.[47]

Table v show that the ability of the liver of the diabetic rat to synthesize fatty acids and glucose is much less than in the normal rat. Furthermore, insulin in the normal animal increases fatty acid synthesis approximately five-fold whereas it has no significant effect in the diabetic. Deficiency of fat synthesis thus became established as a major metabolic defect of the diabetic. Stetten and Klein (1946)[48] did not conclude that this defect was a primary one in diabetes. Subsequent work by others makes it apparent that fat synthesis fails when there is inability, as in the diabetic or starved animal, for intermediaries of the glycolytic or Krebs cycle to be derived from glucose.

The inability of the liver of the diabetic to synthesize fatty acids from glucose, acetate and lactate has been well established by experiments with liver slices and labeled nutrilites. Typical evidence of this type is shown in Table vi which gives representative data from some experiments of Chernick and Chaikoff (1950).[6] The liver from alloxanized rats fails completely to incorporate the labeled glucose into fatty acids. Prolonged insulin treatment prior to sacrifice of the rat for the preparation of the liver slices is required for the restoration of fatty acid synthesis. It might be re-emphasized here, as referred to in the previous discussion of the

paper of Renold, Hastings, Nesbett and Ashmore (1955),[33] that, whereas the addition of insulin to normal rat liver slices results in increase of fatty acid synthesis of acetate, no such insulin effect has been demonstrated unequivocally by the mere addition of insulin

TABLE V
LIPOGENESIS FROM GLUCOSE DETERMINED BY DEUTERIUM FEEDING[48]

	Deuterium in Fatty Acids (per cent of D in body water)	
	Liver	Carcass
Diabetic	2.7	1.2
Diabetic + insulin	1.4	1.1
Normal	6.6	0.8
Normal + insulin	26.1	2.3

TABLE VI
CONVERSION OF C^{14}-GLUCOSE TO FATTY ACIDS BY ALLOXANIZED RAT LIVER SLICES[6]

	Per cent C^{14}-Glucose per Gm. Liver as Fatty Acids
Untreated	0.00; 0.02
Insulin treated	0.37; 0.67; 0.50

to liver slices equilibrated *in vitro*. Prior treatment of some long duration is necessary to bring about such insulin effect.

RELATIONSHIP BETWEEN CARBOHYDRATE AND FAT METABOLISM

There are many experiments in the literature by Lehninger (1951)[25] and others which show that fatty acid metabolism by liver mitochondria can proceed in several directions depending upon the makeup of the system with respect to carbohydrate intermediaries. Thus the experimenter can arrange to have, within certain limits, fatty acid oxidation, fatty acid synthesis or ketone formation predominate. There are experiments on other tissues which show similar interrelations between fatty and carbohydrate metabolism and which have significance in current conceptions of insulin action.

Baker, Chaikoff and Schusdek (1952)[1] reported experiments bearing on this aspect of the insulin problem. (Table VII.) They measured the incorporation of labeled nutrilites into fatty acids by rat liver slices *in vitro*, using rats which had been on various diets for two or three days before the experiment. In the case of the normal rat, glucose or fructose in the prior diet made no

TABLE VII
EFFECT OF DIET ON SYNTHESIS OF FATTY ACIDS BY RAT LIVER SLICES[1]

	Previous Diet	Per cent of Isotope Recovered in Fatty Acid/0.5 Gm. (mean of 4 to 5 rats)	
		Lactate	Acetate
Normal	Glucose	3.3	5.5
Normal	Fructose	5.1	7.7
Diabetic	Glucose	0.5	0.8
Diabetic	Fructose	3.0	3.5

difference in the fatty acid synthesis of lactate or acetate. The alloxanized rats, however, behaved differently. When glucose was the sugar fed the rat liver slices showed markedly diminished fat synthesis from lactate or acetate. In the case of fructose feeding, however, synthesizing ability was essentially that of normal. It is to be noted that these effects were obtained without insulin. Furthermore, the ability of the liver slice to oxidize glucose was not restored. It appears from these experiments that loss of lipogenesis by the liver of the diabetic is not a primary defect. It appears to be secondary to loss of ability to utilize glucose. Fructose can circumvent the difficulty and furnish products of glycolysis which, by their metabolism, furnish energy for the synthesis of fatty acids. These experiments have commonly been interpreted to mean that in diabetes the action of glucohexokinase is impaired so that glucose cannot be phosphorylated by the hexokinase reaction to enter into the glycolytic schema. On the other hand, fructohexokinase, which has been shown to exist as a separate enzyme distinct from glucohexokinase, is supposed to be unaffected in the diabetic. However, a close examination of the literature, for example, Renold, Hastings, Nesbett and Ashmore (1955),[33] demonstrates that the ability of alloxanized rat liver to metabolize fructose, while not abolished, is significantly lower than in normal rats. In view

of this finding it cannot be maintained that the defect in diabetes impeding the metabolism of glucose is completely non-operative in the case of fructose.

The dependence of fatty acid synthesis from lower fatty acids upon products of glycolysis has been well demonstrated by a number of workers: Brady and Gurin (1950),[4] and Shaw and Gurin (1953).[40] Brady and Gurin found that liver slices from fasted or diabetic animals cannot synthesize fatty acids from labeled acetate, in contrast to corresponding preparations from normal fed rats. Summarized data from the work of Shaw and Gurin are shown in Table VIII.

TABLE VIII
FATTY ACID SYNTHESIS BY EXTRACTS OF LIVERS OBTAINED FROM DEPANCREATIZED PIGEONS[40]

Liver Extract	Isotopic Acetate Synthesized into Higher Fatty Acids. Micromoles/10 Mg. of Fatty Acids
Normal pigeons	0.61
Diabetic pigeons	
Additions to medium	
No additions	0.1
Glycogen	1.0
Fructose-1-6-diphosphate	1.7
Glucose-6-phosphate	0.3*
Insulin	No effect

* Higher results were obtained in later experiments.

To overcome the difficulties of cell permeability and other problems pertaining to entrance of nutrilites into the cell, cell-free preparations of mitochondria from livers of depancreatized pigeons or alloxanized rats were used. These preparations retain active glycolytic properties and also perform all the oxidative reactions of the Krebs cycle. In the presence of compounds capable of glycolysis and oxidation by these mitochondrial suspensions, fatty acid synthesis occurs at a rate comparable to that of the intact liver slices. These compounds were hexose monophosphate, hexose diphosphate and glycogen. These authors also found that ATP and diphosphopyridine nucleotide are necessary for the synthesis. It must be remembered that intact slices from normal fed rats respond to insulin by incorporating more labeled acetate into fatty acids, in contrast to slices from fasted or diabetic rats which do not respond to insulin. These facts, together with the recent experiments of Haugaard and Stadie (1952),[17] indicate that oxidation of glycolytic products furnish the energy (presumably through the formation of ATP) to bring about the synthesis of fatty acids by liver. (Fig. 3.)

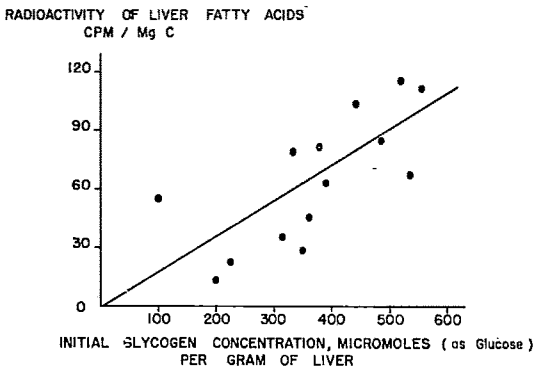


FIG. 3. Association of fatty acid synthesis and glycolysis. The initial liver glycogen was varied by antecedent feeding. The higher the initial value, the greater is the incorporation of isotopic acetate into fatty acids, thus indicating the need for glycolytic products for the synthesis.[17]

CONSIDERATION OF THEORIES OF METABOLIC DEFECTS ATTRIBUTABLE TO INSULIN DEFICIENCY

There are many papers in the literature which report experiments seeking to pinpoint a metabolic defect resulting from insulin deficiency or excess of contrainsulin factors. These have been numbered in the metabolic schema shown in Figure 1 and are: (1) the permeability or transfer hypothesis, (2) the glucohexokinase hypothesis, (3) the hypothesis implicating deficiencies of high-energy phosphate formation, and (4) failure of key oxidative reactions, particularly of the Krebs cycle. Space does not permit a detailed discussion of all the evidence available in this field. An attempt will be made to evaluate critical evidence in relation to the aforementioned hypotheses.

1. Permeability or Transfer Hypothesis. This postulates that, at cell surfaces or perhaps at cell-particulate interfaces which separate enzyme systems one from another, there are barriers (either of a physical or a chemical character) that control the entrance of metabolites to the sites of enzyme action. These barriers may be altered by imbalance of hormones such as insulin (alone or associated with pituitary or

adrenal factors) so that impairment of transfer is altered.

Representative data reported by Levine and his coworkers (1950),[26] who were the first to advocate the permeability or transfer theory, are shown in Figure 4. Eviscerated-nephrectomized

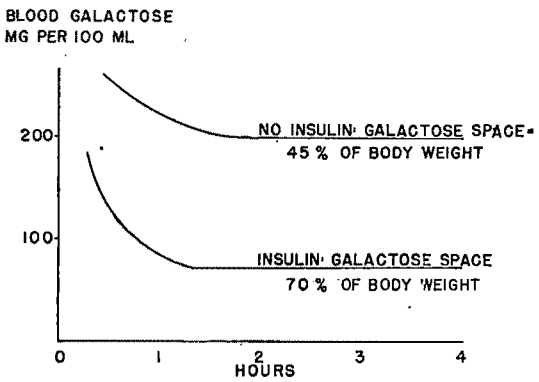
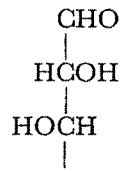


FIG. 4. Permeability theory of insulin action. A metabolically inert sugar (galactose) was injected into eviscerated-nephrectomized dogs and the blood galactose levels followed. Insulin injection markedly increased the "galactose space" indicating its action on "transfer of sugar" across cell barriers.[13]

animals were used into which were injected galactose intravenously. Within a comparatively short time after the injection blood galactose levels became constant, and a calculation of the volume of distribution of galactose could be calculated since, in the type of preparation used, galactose is not metabolized. Without insulin, as the illustration shows, the galactose space was approximately 45 per cent of the weight of the preparation. But following insulin injection this increased regularly to about 75 per cent, which represents body water. These results were interpreted by the authors to mean that there is a barrier which normally excludes glucose from the considerable portion of the body space but under the action of insulin this barrier is lifted. The mechanism by which insulin produces this effect is unknown. In order to avoid implications as to mechanisms, Levine et al. (1950)[26] advocated that the mechanism be termed a "transfer mechanism" rather than a "permeability mechanism." These authors performed similar experiments using a variety of sugars of different configurations. They found that some were "insulin-responsive" whereas others were "insulin-non-responsive." The insulin-responsive sugars had a common configuration in carbon 1, 2 and 3, namely:



Later Park (1953)[30] used isotopic glucose under appropriate conditions and showed that the injection of insulin was followed by an increase of "free" glucose in the heart, skeletal muscle and diaphragm of the rat:

Glucose in tissues
Extracellular distribution..... No insulin
Distribution increased four- to fivefold..... With insulin

Mirsky and his coworkers (1953)[15] performed similar experiments studying the rate of penetration of various sugars into the rat diaphragm *in vitro*. They also concluded that one action of insulin was to accelerate the entrance of certain sugars into muscle. Confirmatory evidence of the transfer hypothesis was reported by Ross (1951 and 1952).[35,36] Ross studied the rate of transfer of glucose across the blood-aqueous barrier in the eye of the normal alloxanized rabbit. He obtained his experimental results by maintaining the blood sugar of the rabbit at elevated levels by appropriate injection and then measuring the rate of increase of glucose concentration in samples of aqueous humor obtained from time to time from the anterior chamber. These data enabled him to calculate velocity constants expressing the rate of transfer of glucose across the barrier of the ciliary bodies. In contrast to the normal rabbit, the velocity constants of glucose transfer were significantly diminished in the alloxanized rabbit. Ross also found that under the action of injected insulin the rate of transfer of glucose across this cell barrier increased.

The evidence here presented seems impressive in indicating that insulin is concerned with the passage of glucose across cell boundaries. The manner in which this effect is achieved remains unknown and the precise significance of a possible decrease in permeation of glucose into tissues as a factor in diabetes mellitus remains to be determined. As will be discussed subsequently, the demonstration of insulin effects upon glucose transfer makes it difficult to evaluate certain evidence which has hitherto been cited to support the hexokinase theory of insulin action.

2. Hexokinase Theory of Insulin Action. Consideration of the metabolic schema shown in Figure 1 indicates that interference with the catalytic action of glucohexokinase results in diminished formation of glucose-6-phosphate. As a result, the entrance of glucose into the glycolytic cycle would be inhibited, the formation of glycogen would decrease, the oxidation of glucose would be diminished and, indirectly, there would be serious interference with the further metabolism of fat and protein. In other words, all of the metabolic derangements observed in diabetes mellitus could be explained upon the basis of an impairment of the hexokinase reaction in mammalian tissue. The hexokinase hypothesis of the diabetic defect therefore becomes a very attractive one and has received much discussion in the literature.

To obtain unequivocal evidence supporting the hexokinase hypothesis, systems must be used in which cell boundaries or cell-particulate interfaces between nutrilites and enzyme systems have been eliminated by appropriate means. Otherwise the effects observed could be equally well attributed to transfer or permeability changes brought about by hormonal action. The demonstration of a differential effect of hormones upon sugars other than glucose does not overcome this difficulty since it is always possible that hormonal effects upon transfer or permeability have a degree of specificity dependent upon the constitution of the sugar. Indeed such evidence is already in the literature.

Attempts to demonstrate altered hexokinase reactions in systems devoid of cellular barriers, for example extracts obtained from muscles of normal and diabetic rats, have been reported in the literature. The diminution of hexokinase activity reported was attributed to an excess of pituitary or adrenal factors. In this situation the addition of insulin *in vitro* was presumed to release the inhibition brought about by the adrenal and pituitary factors. An evaluation of the evidence in the literature, however (Stadie, 1954[41]), failed to substantiate these early claims. A summation of the evidence indicates that insulin, pituitary or adrenal factors, singly or together, have no influence upon the hexokinase reaction when this is determined in cell-free tissue preparations from normal or diabetic animals. It is worthwhile noting that the early equivocal evidence is frequently cited in the literature in support of the hexokinase hypothesis.

This negative evidence with extracts, however, does not necessarily rule out the possibility that in the intact animal or intact tissues *in vitro* it might still be possible to demonstrate a defect in the hexokinase reaction associated with the diabetic state. Two experimental approaches have been used for this type of study: The first is based upon the demonstrated fact that liver and muscle have separate hexokinases specific for glucose and fructose (glucohexokinase and fructohexokinase). If these hexokinases are not only specific with respect to their substrates but are also different in their response to insulin, for example, if glucohexokinase is insulin-sensitive and fructohexokinase is not, it would be expected that the metabolism of glucose would be affected in the diabetic state whereas fructose metabolism would be within normal limits. The second method of studying the hexokinase reaction in intact tissue is based upon the assumption that reactions in the glycolytic cycle beyond formation of glucose-6-phosphate are independent of insulin action and therefore are unaltered in the diabetic state. In consequence, therefore, the study of reactions involving members of the glycolytic cycle would shed light indirectly upon the involvement of the hexokinase reaction. Examples of both types of evidence will be cited.

Cori (1949)[9] discussed experiments in which glucose or fructose was injected into eviscerated rats and the effect of insulin upon the rate of utilization was studied. Typical data from their paper are shown in Figure 5. Sixty minutes after injection of the sugar the animal was sacrificed and the carcass analyzed for residual sugar. The calculated results support the glucohexokinase hypothesis since in the case of glucose injection of insulin markedly accelerated the rate of disappearance of injected glucose so that essentially none was recovered. In the case of fructose no acceleration of utilization was observed. Experiments employing glucose and fructose were reported by Renold, Hastings and Nesbett (1954).[33] In this study liver slices from normal and diabetic rats were equilibrated *in vitro* with the respective sugars. The use of isotopic glucose and fructose enabled the authors to calculate the utilization of these two sugars and the synthesis of glycogen from them. A summary of their data is reported in Table IX. The uptake of glucose was significantly diminished in the diabetic whereas that of fructose was essentially unaltered. However, it is to be noted that the synthesis of glycogen from both glucose

and fructose was markedly diminished. This would indicate that impairment of fructose metabolism also is a factor in diabetes. The authors, however, regard their data as indication that the chief if not the sole defect in diabetes is the impairment of the glucohexokinase reaction.

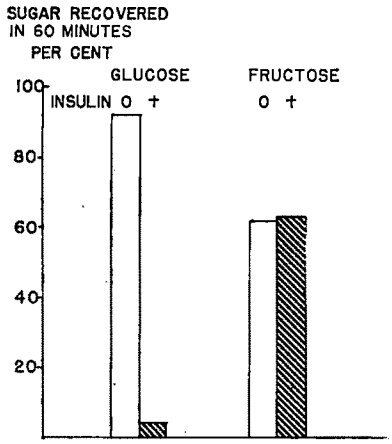


FIG. 5. Hexokinase theory of insulin action. Comparison of the effect of insulin upon the utilization of glucose and fructose by eviscerated rats; residual sugar (per cent of that injected) in the carcass was determined sixty minutes after injection. The increase in the utilization of glucose following insulin injection is assumed to be due to the acceleration of glucohexokinase; in contrast, the absence of effect with fructose is presumably because fructohexokinase is not affected.[9]

Experiments involving this second assumption are illustrated by data (Fig. 6) from a paper by Teng, Sinex, Deane and Hastings (1952).[50] They equilibrated liver slices from diabetic-adrenalectomized and normal rats with glucose or pyruvate. In the case of the diabetic-adrenalectomized rat maximum affects should be observed. They reported that in the diabetic-adrenalectomized rats synthesis of glycogen from glucose was markedly diminished whereas that from pyruvate was essentially unaltered. The authors concluded that "If the hexokinase hypothesis is correct, one would anticipate a decrease in glycogen synthesis from glucose but normal glycogen synthesis from pyruvate. Such indeed are our findings." A similar type of experiment was reported by Chernick and Chaikoff (1951).[7] They studied the metabolism of liver slices from normal and from alloxan-diabetic rats when equilibrated in isotopic glucose or fructose. Both CO_2 production and fatty acid synthesis were determined. The data (summarized in Table x) show that production of CO_2 and of fatty acids from labeled glucose was significantly diminished in the diabetic animal. On the other hand, fructose showed unimpaired production of CO_2 but diminished synthesis of fatty acids. On the basis of these data the authors concluded that the two hexokinases in the liver reacted differently to insulin. That for glucose was impaired by lack of insulin whereas that for fructose was uninfluenced. However, the lack of fructose incorporation into fatty acids in the alloxanized

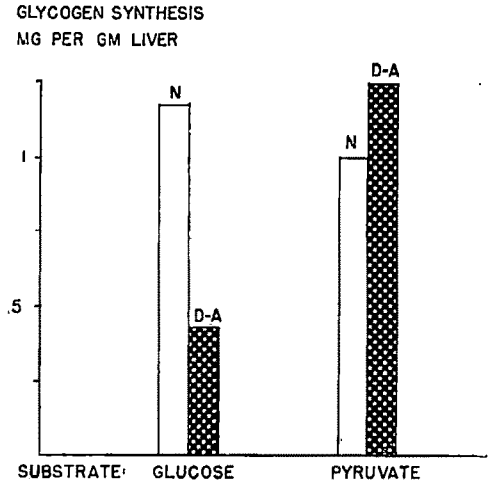


FIG. 6. Hexokinase hypothesis of insulin action; synthesis by liver slices from normal (N) or diabetic-adrenalectomized (D-A) of glycogen from isotopic glucose or pyruvate. Synthesis from glucose by D-A rat liver was depressed; conversion of pyruvate to glycogen was not found to have been impaired in D-A rat liver slices. The authors conclude that the results are in accord with the hypothesis that hexokinase activity in tissues is impaired.[50]

TABLE IX
CONTRAST OF GLUCOSE AND FRUCTOSE METABOLISM BY LIVER SLICES FROM NORMAL AND DIABETIC RATS[32]

	Micromoles per Gm. in 90 Min.	
	Normal	Diabetic
Total uptake		
Glucose	37	14
Fructose	96	99
Glycogen		
From glucose	15	0.3
From fructose	18	2.5

preparation brought the authors to an additional conclusion, namely, that diabetes was associated with a dual block, one of which involved conversion of the 2-carbon-like compound to fatty acids.

Other experiments of these two types have

TABLE X

COMPARISON OF PRODUCTION OF CO_2 AND FATTY ACIDS FROM ISOTOPIC GLUCOSE AND FRUCTOSE BY LIVER SLICES FROM NORMAL AND DIABETIC RATS[7]

	No. of Rats	Sugar Recovered from Slices Per cent			
		Glucose		Fructose	
		CO_2	Fatty Acids	CO_2	Fatty Acids
Normal.....	6	4.0	0.59	6.8	0.82
Diabetic.....	6	1.1	0.02	6.2	0.10

been offered as evidence that the hexokinase reaction in intact tissue is involved in the diabetic state. Can these conclusions be accepted? Only with reserve because evidence for the involvement of a "transfer mechanism" in the diabetic state is so strong that it cannot be excluded as governing the entrance of certain sugars into the sphere of action of enzyme systems. This makes it difficult to determine whether the deceleration or acceleration of metabolic reactions by endocrine disturbances is due to action on specific enzymes such as hexokinase or on some mechanism, still undetermined, which accelerates passage of substrates across cellular barriers.

3. Effect of Insulin Upon Oxidative Phosphorylation. The metabolic schema shown in Figure 1 indicates that the oxidative reactions of the Krebs cycle are concerned with the generation of energy-rich phosphate bonds (ATP, and so on). These bonds are involved in a large number of reactions such as the formation of glucose-6-phosphate by the hexokinase reaction, synthesis of fat and protein, and so on. The hypothesis cannot be excluded that a deficiency in the generation of these energy rich phosphate bonds might be a factor in the disturbed metabolism of diabetes mellitus. Many papers have been published presenting experimental evidence which is interpreted to support this hypothesis.

Early work on phosphate metabolism in the normal and in the diabetic, and the effect of injected insulin thereon, has been reviewed. (Stadie, 1954).[41] Both *in vivo* and *in vitro* analyses have been carried out on liver and muscle in which the various phosphate compounds such as

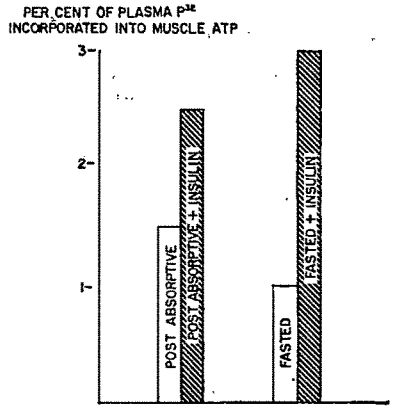


FIG. 7. Oxidative phosphorylation hypothesis of insulin action; the rate of incorporation of P in the blood into ATP of cat muscle following injection of P^{32} and insulin. Samples were taken for analysis three hours after insulin. Glucose was injected initially with the isotopic P in all instances. Insulin significantly increased the rate of turnover of P^{32} in ATP.[37]

glucose-1-phosphate, glucose-6-phosphate, ATP, ADP and inorganic phosphate have been separated and quantitated. The relations among these substances have been determined following the injection of glucose or glucose plus insulin. In addition, the rates of exchange o isotopic inorganic with organic phosphates following glucose or glucose plus insulin injections have been studied. Chief among the experimenters in this field are Kaplan and Greenberg (1944),[20–23] Sacks (1948, 1952, 1953)[37–39] and Haugaard, Marsh and Stadie (1951).[16] The results of these experiments are difficult to evaluate. In general, the evidence would lead one to conclude that some type of interference with the generation of high-energy phosphates is associated with the diabetic state or the action of insulin. On the other hand, none of the evidence enables one to determine unequivocally whether this influence is direct or merely secondary to effects of the general metabolic status of the tissue with respect to carbohydrate oxidation. For example, Sacks (1948)[37] calculated the rate of labile P turnover of ATP following the injection of P^{32}. These

results are indicated in Figure 7. Sacks (1948)[37] concluded that the administration of glucose and insulin brought about an increased rate of phosphate transfer across the cell membrane but he did not speculate as to the mechanism involved. Goranson, Hamilton and Haist

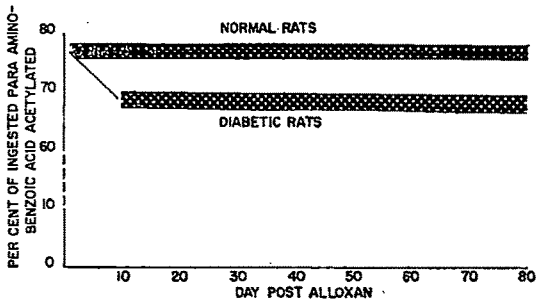


FIG. 8. Phosphorylation hypothesis; presumed impairment of oxidative phosphorylation leading to impairment of acetylation of injected para-aminobenzoic acid by diabetic rats. The hatched area indicates the approximate standard error of the data.[t]

(1948)[14] reported similar observations using tracer phosphates and suggested the possibility that insulin influenced oxidative phosphorylation bringing about accelerated formation of ATP. More direct evidence was reported by Charalampous and Hegsted (1949).[5] They studied the rate of acetylation of a foreign amine (para-aminobenzoic acid) when injected into the normal or the alloxan-diabetic rat.

This acetylation is known to be dependent upon the availability of high-energy phosphate. The results of these experiments (Fig. 8) showed a distinct impairment of the ability of the alloxan-diabetic rat to acetylate PAB. The authors pointed out that this decreased acetylation was restored to normal by the injection of insulin. Another reaction requiring high-energy phosphate, using rat liver homogenate, has also been studied by Foa, Weinstein, Smith and Greenberg (1952).[11] They studied the phosphorylation of thiamine using rat liver homogenates from the normal and from the alloxanized diabetic rat. A summary of their results is shown in Figure 9. In the alloxan-diabetic rat, compared to the normal, there was no diminution of total thiamine but the percentage of thiamine phosphorylated was significantly diminished.

These experiments, together with similar ones reported in the literature, constitute evidence of an indirect nature that high-energy phosphate formation is involved in the diabetic state. The first recorded evidence of a more direct character on this aspect of insulin action was reported by Stadie and Vester (1955).[46] The reactions of oxidative phosphorylation appear to be limited to the mitochondria. Therefore the comparison of the behavior of mitochondria from

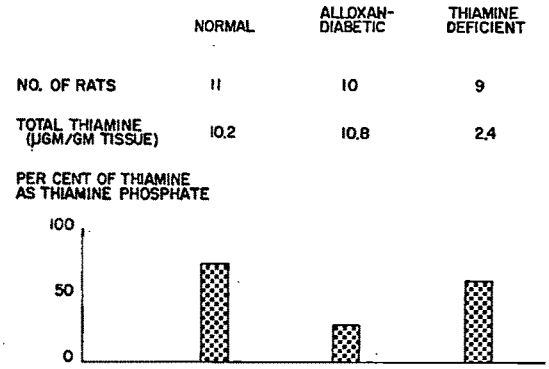


FIG. 9. Oxidative phosphorylation hypothesis of insulin action; diminution of phosphorylated thiamine in livers from alloxanized-diabetic rats.[11]

normal and diabetic animals with respect to ATP synthesis would constitute a fairly direct measurement of possible impairment of oxidative phosphorylation in diabetes. The mitochondria were obtained by the customary differential centrifugation of homogenates prepared from the liver of the cat. The washed

TABLE XI

OXIDATIVE PHOSPHORYLATION* BY LIVER MITOCHONDRIA PREPARED FROM LIVERS OF NORMAL AND DEPANCREATIZED CATS[48]

Cat No.	Pyruvate		β-Hydroxybutyrate	
	Normal	Diabetic	Normal	Diabetic
1	60	7	31	15
2	71	18	42	34
3	52	13	30	5
M	61	13	34	18

* Data gives rates of formation of adenosine triphosphate (μm per minute per mg. of mitochondrial protein) in presence of pyruvate or beta-hydroxybutyrate.

mitochondria were suspended in a buffer solution containing phosphate, pyruvate or beta-hydroxybutyrate. The high-energy phosphate formed as ATP was trapped as glucose-6-phosphate by combination with glucose catalyzed by added yeast hexokinase. The data (Table XI)

show that mitochondrial preparations derived from the liver of depancreatized cats were significantly deficient in their oxidative phosphorylation when pyruvate or beta-hydroxybutyrate were used as sources of energy. Simultaneous measurements of oxygen consumption made it possible to calculate the P:O ratio, which may be taken as a measurement of the efficiency of oxidative phosphorylation. This also was diminished in systems of mitochondria from diabetic cats. A later series of experiments of a similar character confirmed these preliminary observations. This direct indication of the impairment of oxidative phosphorylation observed in the liver of the depancreatized cat requires further exploration before its full significance can be evaluated.

4. Effects of Insulin on Oxidative Reactions in the Krebs Cycle. The possibility that the Krebs oxidative cycle may be a site of action of insulin is indicated in the schema of Figure 1. A number of papers have appeared in the literature supporting this possibility. Krebs and Eggleston (1938)[24] studied the effect of insulin upon oxygen uptake of mince of pigeon breast muscle. The type of data they reported is schematized in Figure 10. The oxygen uptake was measured in the presence and absence of insulin. They found that when catalytic amounts of members of the Krebs cycle (for example, citric acid or succinic acid) were present a typical effect was obtained. This consisted of prolongation of the ability of the muscle mince to take up oxygen. No significant acceleration of the oxygen uptake took place during the initial period but oxygen uptake with insulin continued for a much longer period than without, so that the total oxygen uptake might be doubled. The authors concluded from this type of experiment that "insulin is a catalyst concerned with one of the stages of citric acid cycle." These experiments with pigeon breast muscle have been confirmed. However, muscle from rabbits, cats and dogs appeared not to exhibit the phenomenon. Attempts to show a difference of behavior of normal and depancreatized cats were unsuccessful (Stadie, Zapp and Lukens, 1940[47]), nor was any effect of insulin observed in either of these preparations. These anomalous results have served to discredit the significance of experiments with muscle mince in the study of insulin action.

Other effects of insulin upon oxidative reactions have been reported. For example, oxidation of pyruvate by minces of pigeon breast muscle has been shown to be increased by insulin (Rice and Evans, 1943;[34] Stadie, Haugaard and Perlmutter, 1948).[43] The oxidation of pyruvate to CO_2 by rat diaphragm and heart ventricle slices from the alloxanized rate have

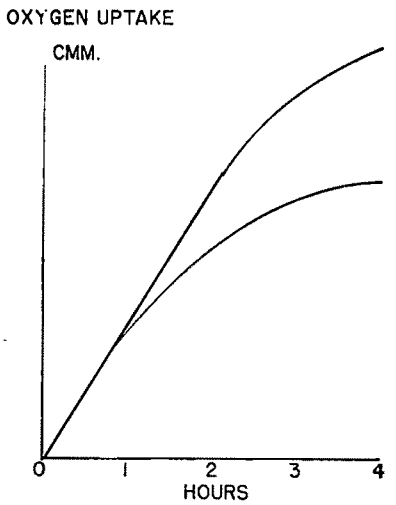


FIG. 10. Krebs cycle oxidations stimulated by insulin; the oxygen uptake of pigeon breast muscle mince + boiled muscle extract and catalytic amounts of citrate ± insulin. Insulin produces a more prolonged uptake of oxygen sometimes doubling the total uptake.[24]

been reported by Hastings et al. (1949).[31] They concluded from their experiments that there is "diminished ability of cardiac and diaphragm muscle to carry out the steps required to convert pyruvate to CO_2."

It is obvious from a consideration of the experiments discussed that a decision from the four possibilities of insulin action here outlined is impossible now. The situation is confused and alternative hypotheses rival each other in the weight of evidence massed for their support. In addition, it would be possible to collect other experimental evidence which would not fit into any of these hypotheses. It is possible that a synthesis of the conflicting evidence will be forthcoming in the near future which will resolve these contending difficulties.

RELATION OF INSULIN TO FAT AND PROTEIN METABOLISM

It is outside the scope of this review to discuss this area in detail. The summation of evidence indicates that there is no direct action of insulin

upon fat or protein metabolism. Disturbances of the metabolic pathways in question appear to be the indirect results of faulty carbohydrate metabolism, already discussed.

COMBINATION OF INSULIN WITH TISSUES

A brief discussion of this phenomenon is pertinent in any review of insulin action. It is well known that substrates combine with enzymes before they undergo chemical reaction. This phenomenon is formulated in the Michaelis-Menten concept which was well reviewed by Clark (1952).[8] Indeed, it would be difficult to imagine how the chemical reactivities of substrates could be influenced unless they came within the molecular distance of enzymes. Otherwise action at a distance would have to be invoked. A similar relationship must hold for the action of hormones upon enzymatic systems. The hormone must approach within molecular distance of the enzyme, otherwise it would be difficult to conceive how the hormone could affect enzymatic action. It is possible to conceive that this close approach of hormones to tissue would be of such nature that it could be characterized as a combination of a chemical character. At all events, a phenomenon first reported by Stadie, Haugaard, Marsh and Hills (1949)[42] was interpreted to indicate the possibility of such a combination of insulin with tissue. A rat diaphragm was momentarily dipped into a solution containing insulin and then thoroughly washed in saline. It was then equilibrated with glucose (no insulin being added) and after a suitable time was analyzed to determine the synthesis of glycogen. Compared to a hemidiaphragm from the same rat not treated with insulin, a marked increase in glycogen synthesis was invariably observed. This was interpreted to mean that during the momentary exposure to insulin the tissue had combined with it so firmly as to resist the disassociating action of washing. This combined insulin retained its customary metabolic effect on the diaphragm by increasing the uptake of glucose and the synthesis of glycogen. In later experiments Stadie et al. (1952 and 1953)[44,45] measured accurately the amount of insulin bound by using insulin tagged with iodine-131 or S-35. It was found that the metabolic activity of the bound insulin was proportional to the amount bound. In addition, the bound insulin was influenced by the hormonal state of the animal, being decreased in the alloxanized animal, in the rat injected with growth hormone or cortisone singly or together, and markedly increased in the case of the hypophysectomized rat. Further study of this phenomenon is necessary before complete evaluation is possible.

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Clinico-pathologic Conference

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STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, B. M. (No. 228986), a sixteen year old white schoolboy, was admitted to Barnes Hospital on November 9, 1953.

Five months before admission upon his return from a visit to California he was told by his family that his skin was pale. However, he felt well until two months before admission, at which time he complained of weakness, easy fatigability and malaise. He was seen by his local physician who gave him several medications, but weakness and fatigability continued. One month before admission the patient was referred to another hospital. Studies there were as follows: red blood cell count, 880,000 per cu. cm.; white blood cell count, 2,700 per cu. cm. The differential count revealed the following: juveniles, 2 per cent; band forms, 6 per cent; segmented forms, 6 per cent, and lymphocytes, 89 per cent. Hemoglobin was 12 per cent of normal. A bone marrow specimen was found to be markedly hypoplastic and a diagnosis of aplastic anemia was made. The patient was given twelve blood transfusions and was referred to the Barnes Hospital. There was no history of drug ingestion, bleeding manifestations, lymphadenopathy or of central nervous system symptoms. The patient had worked as a farm helper for two years and on several occasions he had been exposed to insecticides used for spraying fruit and animals. Among these were DDT, chlordane, and lindane.

Past history and family history were noncontributory.

Physical examination on admission revealed the patient's temperature to be 38°C.; pulse, 92; respirations, 16, and blood pressure, 140/90. The patient appeared chronically ill. The skin was pasty, but the color of the mucous membranes was good. Many small petechiae were scattered over the lower extremities. No icterus was present. Four spider hemangiomas were seen on the trunk. A few tiny anterior and posterior lymph nodes were palpable but no generalized lymph node enlargement was noted. The conjunctivae were clear. The fundi were normal except for one small hemorrhage. The tonsils were enlarged with a small amount of whitish exudate and several areas of hemorrhage on each. A few petechiae were present on the hard palate. There was ecchymosis and slight tenderness over the site of the previous sternal marrow aspiration. The lungs were clear. The heart was of normal size; a loud blowing systolic murmur was heard over the entire precordium. The liver was felt 6 cm. below the right costal margin and was non-tender. The spleen was not felt. Both testes seemed decreased in size, but the genitalia were normal. Rectal examination was not remarkable. Results of the neurologic examination were negative except for a left internal strabismus.

Laboratory data on admission were as follows: red blood cells, 4,420,000 per cu. mm.; hemoglobin, 14 gm. per cent; white blood cells, 4,400 per cu. mm. The differential count revealed the following: band forms, 2 per cent; segmented forms, 4 per cent; monocytes, 2 per cent, and lymphocytes, 92 per cent. Hematocrit was 46 per cent, and the indices were normal. Urinalysis showed the following: specific gravity, 1.030; reaction, 5.5; protein, negative, and

microscopic, normal. Examination of the stool revealed a negative guaiac test. Cardiolipin was negative. Blood chemistry was noted as follows: non-protein nitrogen, 22 mg. per cent; cephalin cholesterol flocculation, 3+; thymol turbidity, 2.8 units; total bilirubin, 1.1 mg. per cent; bone marrow, fatty marrow; the myeloid and erythroid series were markedly depressed. Many tissue basophiles were present. No megakaryoctes were seen. The findings were thought to be compatible with toxic depression. Throat culture revealed alpha hemolytic streptococci. Blood cultures gave negative results. Roentgenogram of the chest was within normal limits.

The patient was given penicillin, 800,000 units a day, and 20 mg. of ACTH in 1000 ml. of 5 per cent glucose in water by slow intravenous drip. During the first three days the temperature ranged between 38°c. and 38.5°c. and no change in his status resulted. On the fourth day the temperature spiked to 41.2°c. The area of the initial sternal aspiration was bulging, tense and fluctuant. Antibiotic therapy was increased. He was given 1,000,000 units of penicillin every six hours and 2 gm. of streptomycin daily. The fluctuant area over the sternum was incised, and about 30 ml. of organized blood clot were removed. No pus was found, and culture was sterile. Repeat blood cultures taken at the first temperature elevation also gave negative results. After surgery the temperature was elevated with spikes to 39°c. for three days, after which it gradually fell and became normal in about twelve days.

On the sixth day of hospitalization the patient passed several black stools which were guaiac negative; however, a few days later stool specimens were found to be guaiac positive. The urine contained no red blood cells. The hemoglobin fell slowly. On the ninth day of hospitalization examination of the blood revealed a red blood count of 2,490,000 per cu. mm., hemoglobin of 8.5 gm. and white blood count of 2,250 per cu. mm. Differential count was the same as that previously noted. At that time the patient was given 2 units of whole blood and thereafter required whole blood transfusions to maintain his hematocrit and hemoglobin levels. He continued to improve symptomatically. The area of the hematoma over the sternum slowly healed. On the fourteenth day of hospitalization the patient was given a daily dosage of 300 mg. of cortisone and 1 gm. of oxytetracycline. A repeat bone marrow aspiration on the twenty-ninth day of hospitalization showed no essential change from those previously examined.

The patient was allowed to go home over the Christmas holidays. He returned in two days; there had been no essential change during the interval. Two days after his return, however, he began to complain of severe pleuritic right anterior chest pain. He did not cough, but the temperature had risen to 39.5°c. Examination of the chest revealed no signs of consolidation, but splinting of the right chest was present. A roentgenogram showed a linear infiltration in the right lower lobe, thought to be indicative of pneumonia, associated with a minimal right pleural effusion. The oxytetracycline on which he had been carried for prophylactic reasons was continued but penicillin and streptomycin were added. The temperature remained elevated and the patient continued to have pain in the chest. Repeat blood counts revealed the following: red blood cells, 3,120,000; hemoglobin, 9.3 gm. per cent; white blood cells, 1,300 per cu. mm. The differential count was as follows: segmented forms, 20 per cent; lymphocytes, 80 per cent, and platelet count, 12,480 per cu. mm. Culture of the sputum isolated Friedländer's bacillus and Staphylococcus citreus. In spite of antibiotic therapy the fever continued, as did all symptoms. The patient began to complain of tenderness to light palpation over the anterior right chest. A repeat chest x-ray revealed partial resolution of the right middle lobe atelectasis which was thought to be present in the previous film. It also showed a partial clearing of the pneumonitis previously noted. A patchy destructive process was described at the anterior end of the fourth right rib, although this finding was subsequently questioned by several observers. Cortisone therapy was reinstituted because of the low platelet count, and erythromycin and chloramphenicol were added.

The patient seemed to improve for a short time. His temperature fell for the first time in five days to 37.2°c. but again rose, ranging between 38°c. and 39.5°c. He became pale and weak, his appetite diminished, and he began to bleed from the gums and from a large necrotic ulcer which appeared in the nose. Packs were of only partial benefit in controlling bleeding. The ulcers became more necrotic and continued throughout the remainder of his life. Blood counts continued to fall and the patient required constant blood transfusions. Subsequent roent-

genograms of the chest showed continuing resolution of the pneumonitis and atelectasis of the right middle lobe. More petechiae appeared in the skin. The stools became guaiac positive, and red blood cells appeared in the urine. Proptosis of the right eye was noted and slowly increased. This was not associated with pain, change in vision, or other neurologic signs. Results of lumbar puncture were within normal limits. Friedländer's bacilli were cultured from the sputum on two occasions. Culture thereafter contained non-hemolytic Staph. citreus and coliform organisms. Blood cultures gave negative results. Four days before death the patient began to complain of severe sore throat. The left tonsil was found to be large, red and boggy, and the next day a white exudate was present. Culture from this exudate showed a heavy growth of coliform organisms. Chloramphenicol therapy, which had previously been discontinued, was re-instituted. On the day before death the patient became apprehensive and dyspneic. The skin rapidly became markedly icteric. The temperature rose to 40°C. and the patient expired on February 9, 1954.

CLINICAL DISCUSSION

DR. VIRGIL SCOTT: The patient for discussion is a sixteen year old schoolboy with bone marrow failure who had been exposed to insecticides, both DDT and lindane, while working on a farm during the summer. The duration of the marrow failure at the time he came to the hospital in early November was at least two months and possibly five months. During the month prior to admission he had received twelve transfusions in another hospital. Abnormalities noted on admission included fever, petechiae, inflammation of the tonsils, systolic apical murmur, enlargement of the liver and large ecchymoses over the sites of the previous sternal bone marrow aspiration.

Laboratory data on admission were remarkable chiefly for the hematologic findings. The bone marrow was fatty with depression of all three of the important elements, the myeloid series, the erythroid series, and the megakaryocytes. Because of recent transfusions the peripheral blood on admission did not reveal any significant anemia, but this absence was short-lived. The white blood cell count varied from a high of 4,600 with 20 per cent granulocytes, to a low of 360 with no granulocytes present. It was implied in the protocol but not so stated that there was persistent thrombocytopenia, the highest platelet count being recorded as 44,000 on the third day of hospitalization. On the fourth day his temperature, which had been between 38°C. and 38.5°C., rose to 41°C. Meanwhile, ACTH and prophylactic oxytetracycline were given. The cause of this initial fever was uncertain. Possibly it was due either to the pharyngitis or to the fluctuant site of the sternal marrow aspirations done elsewhere, although cultures of this material gave negative results. In any event, with administration of penicillin and streptomycin added to that of oxytetracycline the temperature gradually returned to normal over a twelve-day period. During the next five weeks he remained afebrile, receiving prophylactic oxytetracycline and penicillin plus cortisone. No evidence of bone marrow activity was found. He was allowed to go home over the Christmas holidays while taking the drugs and returned after two days. Two days later the final stage of his illness began with fever and right pleural pain. Friedländer's bacilli were cultured twice from the sputum. Penicillin and streptomycin were again added to the oxytetracycline, and almost all available antibacterial agents were used subsequently with some success, at least from the standpoint of the pulmonary lesion. Meanwhile, only a few days after the onset of pulmonary difficulty, epistaxis began which required nasal packing. Infection developed at this site and progressed until the time of his death one month later. Bleeding from this and from other sites constituted a real problem. Three days after the onset of the cellulitis of the nose proptosis of the right eye was detected, and the possibility of sinus thrombosis was raised. Results of neurologic and spinal fluid examinations were normal. The patient remained febrile with a spiking temperature without evidence of a localized infection other than the aforementioned pulmonary lesion and cellulitis of the nose. Arthralgia and myalgia were thought to be the result of transfusion reactions. Four days before death pharyngitis recurred; coliform organisms were recovered from the pharynx, and on the day before death jaundice appeared. May we see the x-rays?

DR. GLADDEN V. ELLIOTT: The pertinent radiographic examinations were limited to the chest, of which there were several examinations made prior to his death. The initial examination on his first admission to the hospital on November 10 served as an excellent base line; it was

within normal limits. The first abnormalities were noted on his return to the hospital after the Christmas holidays. The examination on December 29 showed an obvious dense localized discrete infiltration of the right lung base which in the lateral view was somewhat difficult to see, but probably occupied primarily the middle lobe. The diaphragm on the right side was markedly elevated. A little pleural effusion was noted in both the anterior and posterior gutter. Over the next month there was a slow but incomplete resolution of this pneumonic process with persistence of some shrinkage or atelectasis of the middle lobe segment. During the month a destructive process in the anterior end of the fourth rib was questioned several times. Our final film was a laminagram exposed on January 15 in an attempt to further delineate this fourth rib anteriorly. At the time of this laminagram no apparent involvement of that fourth rib was noted. The cortices appeared intact throughout.

DR. SCOTT: Dr. Loeb, before I ask Dr. Shank to review the toxicology of the insecticides to which the patient had been exposed, would you further define the nature of the hematologic problem and mention something of the correct terminology which should be employed in such instances.

DR. VIRGIL LOEB, JR.: If one is confronted by a patient presenting the clinical story, as did this boy, of weakness, easy fatigability, fever without obvious infection, bleeding tendency, and laboratory evidence of anemia, thrombocytopenia, and granulocytopenia, immediately one would think of two diagnoses, either acute leukemia or bone marrow failure. The diagnosis of acute leukemia was ruled out early in his illness by the finding of hypoplastic bone marrow and by the absence of peripheral lymph node enlargement. The term "bone marrow failure" is applied here simply as a generic term meaning that the bone marrow fails to produce its normal complement of cells; in this case all three cellular types were affected, resulting in the absence of red cell production, granulocyte production and platelet production. In this particular case, since the bone marrow was actually morphologically aplastic, the term "aplastic anemia" can be applied. It is in those cases where the same peripheral blood picture is noted and the bone marrow appears normally cellular that the term aplastic anemia is incorrect, and for that reason the term "bone marrow failure" is preferable. The cases of aplastic anemia may be divided into main etiologic categories, on the one hand those due to an endogenous or idiopathic cause for which no known etiology can be demonstrated or, on the other hand, those due to an exogenous cause, in most cases a chemical, a drug, irradiation or some physical agent. Certainly the picture following irradiation or exposure to radioactive isotopes is comparable to the situation in this case, but the most likely diagnosis here would be based on past history of exposure to a known bone marrow toxin, either DDT or lindane.

DR. SCOTT: With respect to the term "aplastic anemia," isn't it true that the anemia is the least important of the difficulties, red cells being reasonably easily replaced, whereas the absence of white cells and platelets is more difficult to manage.

DR. LOEB: From the standpoint of therapy, perhaps anemia is the least difficult to manage; certainly when the platelets and granulocytes are decreased one runs into one complication after another, such as those manifested in this case.

DR. SCOTT: Dr. Shank, would you review briefly the toxicology of these two insecticides?

DR. ROBERT E. SHANK: This patient was exposed to and had used DDT and lindane during two summers, first in the summer of 1952, then more recently in the summer of 1953. It became important to examine the possible relationship of his exposure to the occurrence of pancytopenia. In reviewing the literature one finds that both DDT and lindane (benzene hexachloride) have been judged to be implicated in the production of thrombocytopenia, agranulocytosis and in the decrease of red blood cell formation.[1,2] Lindane seems particularly culpable in this regard. Much more DDT has been used in the United States and throughout the world than has lindane, but the number of cases reported of pancytopenia resulting from the use of lindane is much greater. We felt that of the insecticides used here, lindane was more likely to be the responsible agent. In examining the history more closely we found that during the summer of 1953 there was one period when

[1] FRIBERG, L. and MARTENSSON, J. Case of panmyelophthisis after exposure to chlorophenothane and benzene hexachloride. *Arch. Indust. Hyg. & Occup. M.*, 8: 166, 1953.

[2] DANOPOULOS, E., MELISSINOS, K. and KATSAS, G. Serious poisoning by hexachlorocyclohexane: clinical and laboratory observations on five cases. *Arch. Indust. Hyg. & Occup. M.*, 8: 582, 1953.

the boy sprayed a large group of cattle with a mixture that contained both DDT and lindane. He used it for about half a day during the morning, then had to leave his work for twenty-four hours. During this period he stated that he was weak, became nauseated and then vomited. The symptoms described are those which might occur with acute toxicity due to lindane. It was not until two months later that he was noted by friends to be pale, and it was even later than that when bone marrow failure was actually noted. I would like to conclude by stating that these insecticides are probably highly toxic for the bone marrow.

DR. SCOTT: The additional information that you have given about his spraying of the cattle was not in the hospital record, and it provides more support for insecticide toxicity than we had. I understand that some *in vitro* studies using lindane were made. Do we have any information about those studies?

DR. LOEB: To the best of my knowledge they were all inconclusive and showed no evidence of an immunologic mechanism responsible for the thrombocytopenia.

DR. SCOTT: Dr. Moore, before we get into the subject of treatment, what can be said about the prognosis in a situation such as this when patients have bone marrow failure allegedly due to some toxic agent, either drug or insecticide? Also, is the appearance of the bone marrow of any value in estimating the prognosis?

DR. CARL V. MOORE: The first thing one can say is that when the white cell count and the platelet count are markedly depressed the prognosis is worse than when those values are reasonably near the normal. Secondly, when the marrow is hypocellular over a period of a week or two, eliminating just a transient hypocellularity, the prognosis is certainly worse. There are recoveries. One does see persons who develop hypocellular marrows from benzene and other toxic agents with severe leukopenia and severe thrombocytopenia who gradually over a period of many months do recover. Those patients, however, certainly are the exception rather than the rule, and probably nothing we do brings about recovery. They simply recover by themselves, as far as we know.

DR. SCOTT: The objectives of treatment then are to prolong life until such time as bone marrow may begin again to function spontaneously?

DR. SCOTT: Such an attempt was made in this boy.

DR. SCOTT: In attempting to achieve these objectives, two main therapeutic approaches were utilized. The first of these was the use of cortisone. Dr. Loeb, I believe you were on hand when the decision to use cortisone was made. Would you tell us what was hoped for by utilization of this agent?

DR. LOEB: The only reason for giving cortisone to this boy was, as Dr. Moore said, to attempt to prolong his life so that he could ultimately recover on his own. There is a well recognized effect of cortisone upon the preservation of vascular integrity in thrombocytopenic states which seems, for some reason not clear to me, to prevent spontaneous bleeding even though the platelet level is low. There doesn't seem to be much evidence that cortisone under conditions of bone marrow depression can stimulate platelet formation, but there is good clinical evidence and some experimental evidence that cortisone will at least prevent some spontaneous bleeding through the capillary wall in thrombocytopenic states. It was for that reason alone that it was felt cortisone therapy was justified, an attempt thereby being made to stop the spontaneous hemorrhages which are usually the cause of death in these cases.

DR. SCOTT: Then there were two aspects for which cortisone was used: first, in the hope that it might in some way stimulate the bone marrow; second, and more important, for its non-specific effect in helping to maintain capillary integrity. Of course, in this instance, as the patient has no granulocytes, the use of cortisone is a two-edged sword. We are all familiar with the fact that patients who are receiving cortisone, for reasons not entirely clear, are prone to infections of one kind or another.

Dr. Wood, we know that you are interested in the effects of cortisone experimentally upon leukocytes. I wonder if you would summarize briefly for us what these effects may be. And, since this patient had so few granulocytes, would you express an opinion as to whether cortisone would have been any more detrimental under these circumstances. In what other way does cortisone effect the defensive processes of the body?

DR. W. BARRY WOOD, JR.: We have been interested in studying the effect of cortisone on infections. It is clinically well-known that patients on cortisone therapy tend to acquire infections. Some of our earliest experiments were done in Dr. Glaser's laboratory with streptococ-

cal pneumonia and we were able to show that cortisone would depress the inflammatory reaction in the lung. What you see in that situation is the failure of the leukocytes to penetrate into an infected area in the large numbers that characterize their behavior in the normal animal. In other words, there is an anti-inflammatory effect of cortisone. But once the leukocytes get to the infected area they do a good job of destroying bacteria. Our experiment suggests that the cortisone acts primarily by preventing the leukocytes from getting into the infected site by the usual mechanism of diapedesis. Dr. Fred Allison has been studying the problem in the rabbit ear chamber to determine in what way cortisone stops the leukocytes from getting into the infected area; he has had to work out a tricky method of producing an inflammation in the rabbit ear chamber in which he can show that cortisone seems to prevent the entire sequence of events ordinarily following inflammatory stimulus. In other words, if cortisone is given first there is no change in the blood vessels, whereas usually the blood vessels dilate at the beginning of an inflammatory response, then the leukocytes stick to the wall of the vessel, crawl through and into the infected area. Cortisone stops all this. Our present idea is that cortisone must work at a very early phase of the inflammatory mechanism and stop it from being set off. What that involves we don't know, because we don't know what sets off an inflammatory reaction.

In answer to your second question about using cortisone in the presence of agranulocytosis, there is no doubt that the hormone is more dangerous to use under those circumstances since the absence of leukocytes is the loss of one of the main body defenses. If one inhibits further the migration of those few leukocytes into the infected area, the situation is certain to become worse. In treating this boy, I am sure everyone realized what was going to happen, and they tried to protect him with antibiotic therapy. At the present time one can't say too much more about the effect of cortisone on infections except to point out that dosage is extremely important. If an experiment is properly set up in the laboratory one can demonstrate that cortisone protects an animal against infection. To do this very small doses of cortisone must be used. If the dosage of cortisone is increased to the range used in this case one gets the anti-inflammatory effect and, of course, less resistance to infection. So, as with many other experiments, either result can be attained, depending upon the dosage. What we have to worry about here, of course, is the big dosage with its anti-inflammatory effect and the greater susceptibility of the patient to infection.

DR. SCOTT: Is the immunologic response in the animal altered?

DR. WOOD: The immunologic response is very difficult to say anything about at the moment because of the conflicting evidence. There are those who have demonstrated that antibody production can be depressed by the use of cortisone and ACTH. Other reliable investigators have shown that that is not true of certain antigens, that antibody production is just as great with cortisone. The latest information is that cortisone will depress antibody production, depending upon the size of the antigen; if the antigen is large enough, cortisone will depress antibody production, whereas with relatively small antigens, such as polysaccharides obtained from bacteria, cortisone has no effect on antibody response.

DR. SCOTT: One of the objectives of treatment with cortisone, then, was the prevention of bleeding; this in the final analysis was not very successful. The second objective of treatment was the prevention of infection, and for this purpose penicillin and oxytetracycline were utilized prophylactically. Dr. Glaser, you are interested in penicillin prophylaxis under somewhat different conditions in the normal person rather than in the person without granulocytes. What kinds of infection can one hope to prevent with penicillin?

DR. ROBERT J. GLASER: There are some that one can prevent with penicillin, particularly hemolytic streptococcal infection, probably pneumococcal infections and, less predictably, staphylococcus infections, although these have become very resistant to penicillin in a large percentage of cases. Little can be done with any of the gram-negative rods or with any other microorganisms. Penicillin is a fairly limited tool and ought to be used under restricted circumstances.

DR. SCOTT: Do you think it should have been used under these circumstances?

DR. GLASER: In this case one had to give the patient the benefit of the doubt. Here, of course, much larger dosage was used and rightly so.

DR. SCOTT. Dr. Harford, what about the experience with oxytetracycline? Is the experi-

ence, using this agent prophylactically, extensive enough now to predict what can and what cannot be prevented with this agent?

DR. CARL HARFORD: There is not enough information to be able to make an accurate statement on that point.

DR. SCOTT: In early January, then, the patient developed pneumonia and Friedländer's bacilli were cultured from his sputum on two occasions. On both occasions the reports state that a heavy growth of Friedländer's bacilli was obtained and that the organism killed a mouse. Is that good evidence that the pneumonitis present was caused by this agent?

DR. HARFORD: It is fairly strong evidence, but it is not conclusive, because heavy growth of similar organisms may be obtained from the respiratory tract of persons who have no clinical manifestations of respiratory infection.

DR. SCOTT: Will any Friedländer's bacillus kill a mouse?

DR. HARFORD: That takes us back to the matter of classification. We have chosen to use the term to mean an encapsulated gram-negative coliform bacillus which can kill a mouse; simply because of the importance of capsules in virulence, we think that point has clinical significance, although people who spend time classifying bacteria might not agree, on the basis of such evidence, that this was Friedländer's bacillus.

Dr. Scott: So part of our evidence here that this was Friedländer's bacillus was that it killed a mouse. What about the sensitivity of this organism to oxytetracycline?

DR. HARFORD: The organism grew in 50 μg. per ml. of polymyxin. It grew in 10 ml. of oxytetracycline with partial growth at 20 and 30 μg. So it was moderately sensitive under the conditions of this test.

DR. SCOTT: The other infection which developed was the cellulitis of the nose; from this nothing of significance was cultured. A light growth of proteus and a moderate growth of staphylococcus albus were present which were non-hemolytic and coagulase-negative.

This patient was in the hospital for ninety days. He received eighty-five days of steroid treatment and a total of 198 days of antibiotic treatment, including sixty-three days of oxytetracycline, forty-nine days of parenteral penicillin, three days of tetracycline and twenty-three days of chloramphenicol. For fifteen days he received one antibiotic, for forty-six days two antibiotics, for twenty-five days three antibiotics, and for three days four antibiotics. For almost a third of his hospital stay he was receiving more than two antibiotics each day. Now, Dr. Reinhard, since chloramphenicol is an agent which has been described as producing this type of hematologic difficulty, do you think it was justifiable treatment in this circumstance?

DR. EDWARD REINHARD: This is a problem that comes up many times, and one has to keep in mind that medicine is very often a question of analyzing statistical probabilities. This man had a very serious illness which certainly constituted an immediate and desperate threat to his life. He had a severe infection, and the chances of his succumbing to this infection were great. The probability that he would be sensitive to chloramphenicol was no greater than the chance of anyone else being sensitive to it. Probably in not more than one of several hundred thousand people receiving chloramphenicol will aplastic anemia develop due to this drug, so that if he had had an infection more sensitive to chloramphenicol than to any other available antibiotic, it should by all means have been used.

DR. SCOTT: Is there any evidence that the duration of chloramphenicol treatment has anything to do with the development of this type of reaction? Can a reaction be evoked as frequently from one day of treatment with chloramphenicol as with a month's administration?

DR. REINHARD: The same principles that apply to other drugs probably apply here. If a patient had had previous chloramphenicol therapy he might have a severe blood reaction of this sort from a single tablet; if, on the other hand, he had never received the drug it would probably require a week or ten days before such a reaction would take place, if it took place at all.

DR. SCOTT: Now we come to the terminal part of the illness. You will recall that he remained febrile despite all these antibacterial agents. His bone marrow showed no response. Infections in the nose persisted. The pulmonary lesion apparently did not progress. One aspect of his illness about which we have little information is the fact that jaundice developed on the day before he died. Dr. Shank, what about the jaundice? Do you think this was homologous serum hepatitis or widespread bacterial infection of the liver. Is the information we have too meager to allow us to guess?

DR. SHANK: When the patient was admitted to the hospital his liver was described as large but non-tender. Yet at that time liver function tests included a cephalin flocculation of 3+, a thymol turbidity within normal range and a total serum bilirubin that was just 1.1 mg. per cent. Since this involvement developed so late, relative to the findings described earlier, it is a little difficult to assess. I suppose it should be stated that he might have had homologous serum jaundice. On the other hand, he might have had some form of hepatic disease on admission; both of the insecticides to which he had been exposed do, on chronic exposure, produce hepatic damage. Little laboratory evidence of that type of disease was found. I am afraid I cannot go further in giving you a specific answer to your question.

DR. SCOTT: Dr. Loeb, do you think there was sufficient bleeding into the skin and internal organs to account for the development of jaundice in this patient.

DR. LOEB: I have no basis on which to say yes or no. I think it is a good possibility, especially if the liver was jeopardized and could not clear the bilirubin due to increased hemolysis.

DR. REINHARD: I would say that the jaundice here was probably due, as has been previously stated, to a combination of at least two causes: first, a certain amount of liver damage probably present at the time of admission to the hospital which could have been due to exposure to hepatoxic chemicals or possibly to the overwhelming infection; second, to the rather extensive hemorrhage occurring in internal organs.

DR. SCOTT: The clinical diagnoses here were (1) aplastic anemia due to exposure to insecticides, (2) pneumonia, right middle lobe and (3) hepatitis due to toxins.

DR. SHANK: It would be well to point out to physicians that there are electrical vaporizers on the market which heat crystals of lindane and chlordane to provide a continuous or intermittent mist of these chemicals. These are commercially advertised as effective home or farm insecticides and are designed to be used on porches or in areas where insects are a nuisance. It should be obvious that such devices constitute a serious health menace,[3] and in my opinion, should be taken off the market.

[3] Health problems of vaporizing and fumigating devices for insecticides. *J. A. M. A.*, 152: 1232, 1953.

PATHOLOGIC DISCUSSION

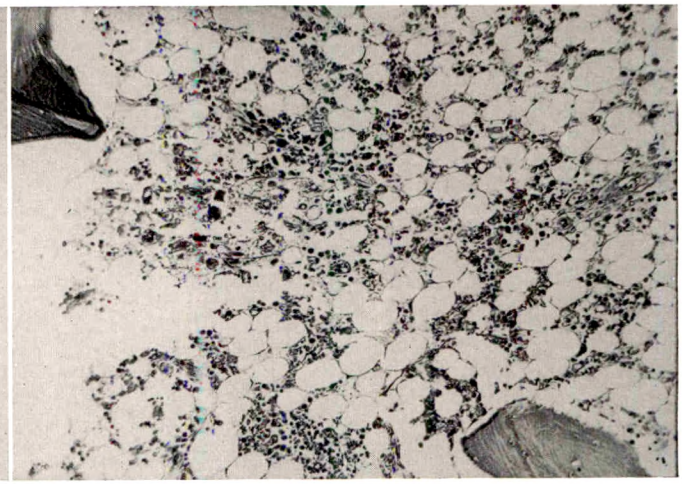
DR. ROBERT W. OGILVIE: At autopsy the skin was icteric. A necrotizing inflammation involved the anterior nares. Numerous petechiae and ecchymoses were scattered through the skin, mucous and serous membranes, lungs, heart, kidneys, testes and retroperitoneal tissues. The bone marrow of the ribs, vertebrae and sternum appeared grossly hypoplastic, while that of the femur and tibia was yellow and fatty. The liver was enlarged, weighing 2,580 gm., and revealed no apparent necrosis or fibrosis. The spleen was of normal size and gross appearance. The cervical, tracheobronchial, periportal and mesenteric lymph nodes were moderately enlarged, firm, discrete and red-brown. Multiple petechiae and firm focal hemorrhages measuring up to 3.5 cm. in diameter were scattered throughout the lungs; however, none of these foci had the usual gross appearance of bronchopneumonia. The middle lobe of the right lung was fibrous and contracted. It was adherent to the anterolateral thoracic wall beneath the right fourth rib. The rib was increased in diameter at this point and the periosteal tissues were thickened, firm and grey with soft, necrotic, and yellow-brown areas. The kidneys were enlarged and weighed 325 and 340 gm. Their parenchyma was pale and contained multiple petechiae. In the pelves bilaterally extensive hemorrhages were noted. A small abscess was present in the cortex of the left kidney.

DR. DAVID E. SMITH: Dr. Ogilvie has described for you the extensive hemorrhagic manifestations present in this case. Figure 1 is a picture of the stomach (gross) to show how impressive some of the mucosal hemorrhages were. A large ulcerated area of gross hemorrhage lay in the lower part of the esophagus, and the mucosa of the corpus and fundus of the stomach down to a sharp line in the pylorus was congested and filled with extravasted blood. The same type of hemorrhage was present in the renal pelves and was represented in parenchymatous organs by ecchymoses in many sites.

A microscopic section of the bone marrow is illustrated in Figure 2. This particular section is from a vertebra; however, it is representative of all sections from the usually hematopoietic marrow. Small foci of granular debris represent active necrosis, and an almost completely exhausted appearance, as far as any single



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FIG. 1. Extensive hemorrhage in the mucosa of the corpus and fundus of the stomach with sparing of the pyloric region. The esophagus in the upper part of the illustration has a large hemorrhagic ulceration in the lower portion.

FIG. 2. Foci of necrosis and generalized hypoplasia of the bone marrow in a vertebra. The remaining cells are plasma cells, reticulum cells, and a few representatives of the granulocytic series, but no megakaryocytes are present.

hematopoietic cell is concerned, characterizes the remainder of the section. The scattered cells remaining are a few plasma cells and representatives of various series, particularly the granulocytes. It is notable that practically no megakaryocytes are present in this or any other section.

The liver is histologically fairly normal. (Fig. 3.) There is a little fatty metamorphosis around the central veins, and a few large hepatic cells suggest a regenerative activity that might be interpreted as evidence of a residual of toxic damage, but nothing justifies a morphologic diagnosis of active viral or toxic hepatitis. The icterus, therefore, seems to have developed on the basis of extensive extravasation of blood in various tissues and widespread infection in many organs, although neither bacteria nor inflammation are apparent in the liver itself.

In a typical lymph node illustrated in Figure 4 there are large colonies of bacteria that are largely cocci. These gram-positive cocci were not identified by culture; however, the blood yielded in addition a profuse growth of colon bacilli and a diphtheroid. The lymph nodes were further remarkable for the presence of edema in the sinusoids and a remarkable hyperplasia of reticulum cells and other large mononuclear cells. Abscesses in the other organs such as the kidneys were also characterized by tremendous growth of bacteria and the lack of any evidence of polymorphonuclear leukocytes in their walls.

The lesion of the right fourth rib was a large subperiosteal abscess and a section from its wall is shown in Figure 5. Here again there can be seen large masses of bacteria and the same type of granulation tissue with a mononuclear exudate instead of polymorphonuclear leukocytes. Above the capsule of the abscess there is a wide collar of recently formed bone that is almost as thick as the normal rib and which apparently has arisen in response to the inflammation in the periosteum.

The final section (Fig. 6) is from the lung. It is representative of the hemorrhagic nodules which were present throughout all lobes. Hemorrhage into the alveoli, large masses of fibrin in the alveolar spaces, and practically no cellular reaction were the predominate features of these nodules. In other sections large numbers of bacteria were associated with this type of lesion, which represented the effects of a bacterial infection of the lung that had led to the breaking down of capillaries and escape of fibrin and red cells into the alveoli without having the usual polymorphonuclear leukocytes present to be called upon to participate in the morphologic response. It can be very appropriately called an agranulocytic pneumonia. The area in the middle lobe of the right lung that corresponds to the shadow in the roentgenogram was an organized pneumonia. It could have resulted from a similar although less severe pneumonia of this atypical type, although in its final form it

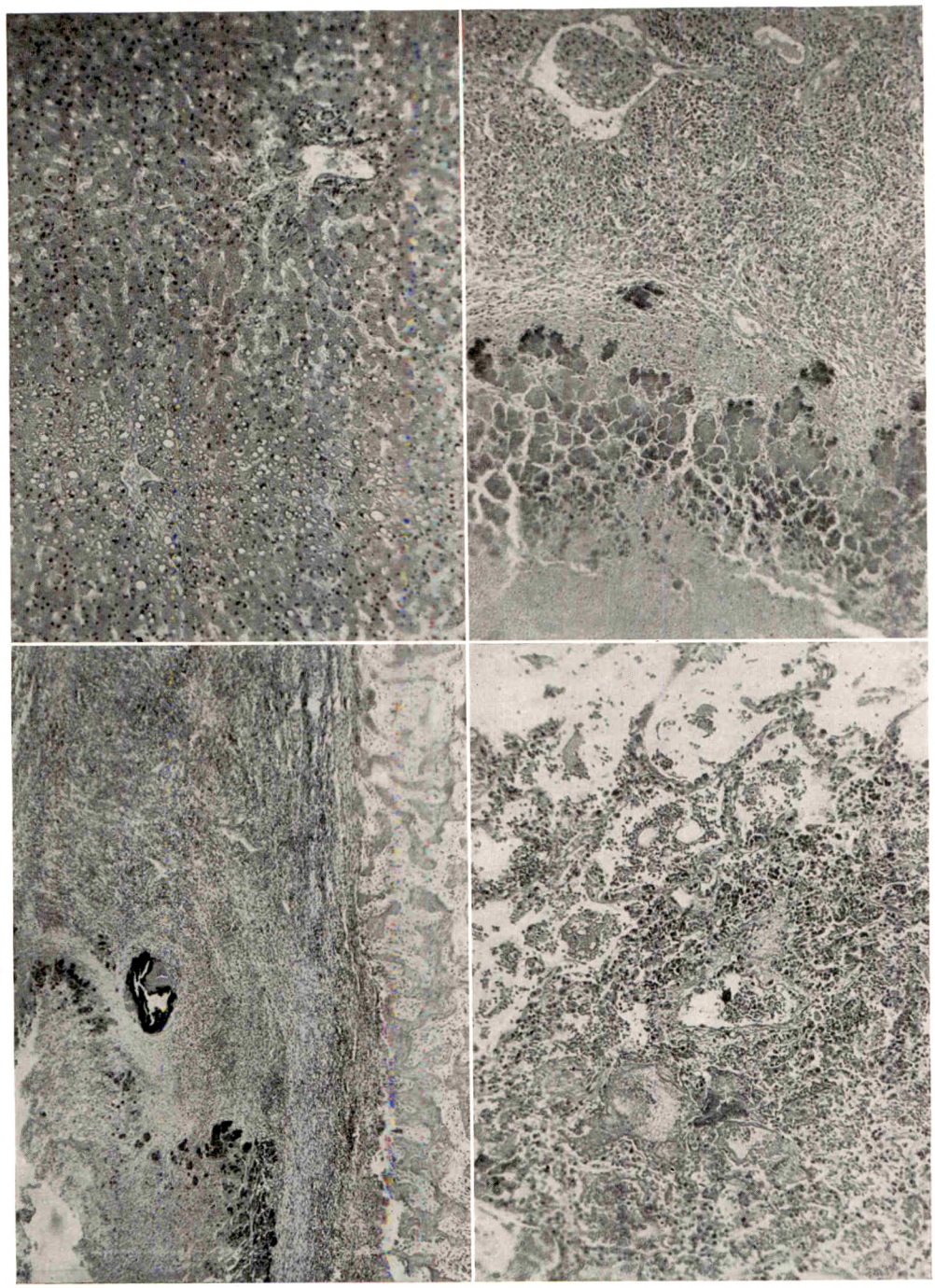


FIG. 3. Centrolobular fatty metamorphosis and atrophy of the liver without necrosis or other evidence of a toxic or infectious hepatitis.

FIG. 4. A cervical lymph node with a large abscess in which there is extensive bacterial colonization and little cellular reaction in the wall. The parenchyma on the left is composed almost entirely of hyperplastic reticulum cells.

FIG. 5. The subperiosteal abscess of the right fourth rib with the same colonization of bacteria and lack of polymorphonuclear leukocytes as those seen in the lymph nodes. Above the abscess is a portion of bone that has been newly formed in response to the irritation of the periosteum.

FIG. 6. An agranulocytic pneumonia with fibrin, hemorrhage, mononuclear cells and bacteria in the alveoli, but no polymorphonuclear leukocytes are present.

was not appreciably different from any other organized pneumonia.

The tissue responses in this patient were primarily an almost total aplasia of the bone marrow and no evidence of regenerative activity or extramedullary hematopoiesis in any organ. These changes have no specific characteristics that would enable one to identify them as the effects of a particular causal agent, but they are perfectly compatible with the changes of toxic aplasia that can occur after such agents as lindane, and the history supplied by Dr. Shank makes it seem quite likely that that was their etiology. In addition there were the effects of an extensive spread of bacteria throughout the body during the last weeks or days of life. This resulted in the formation of abscess walls that were atypical because of the lack of their usual component of polymorphonuclear leukocytes, yet which represented considerable effort on the part of the tissues to combat the infection. Finally, there were extensive hemorrhages that apparently were due to the lack of platelets and to the septicemia and which led to the icterus without specifically involving the liver.

Final Anatomic Diagnoses: Aplastic anemia; septicemia with abscesses in the kidney and periosteum of a rib; reticulum cell hyperplasia of lymph nodes; agranulocytic pneumonia; petechiae, ecchymoses and hemorrhages in all organs.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Research Society Abstracts

Western Society for Clinical Research

ABSTRACTS OF PAPERS PRESENTED AT THE EIGHTH ANNUAL MEETING, CARMEL, CALIFORNIA, JANUARY 28 AND 29, 1955

PRODUCTION OF RENAL CORTICAL NECROSIS WITH SEROTONIN (5-HYDROXYTRYPTAMINE). *Ernest W. Page,* and Mary Beth Glendening.* Dept. Obstetrics and Gynecology, Univ. California School Medicine, San Francisco, Calif.

When the kidneys of rats, mice, guinea pigs or rabbits are exposed under anesthesia the intravenous injection of serotonin causes a visible blanching of the kidneys. In normal rats the ED_{50} for this effect is 0.007 mg. of serotonin base/100 gm. of body weight. This reaction is inhibited by 1-hydrazinophthalazine. Intra-arterial injections of india ink during the renal blanching indicate that the ischemia occurs chiefly in the cortex. The intravenous infusion of 1 mg. of serotonin per hour for five to seven hours to normal 200 gm. rats, followed by sacrifice a week later, produces patchy areas of renal cortical necrosis. The lesions resemble those found at autopsy in pregnant women who die more than twenty-four hours after the occurrence of severe abruptio placentae.

Previous work has indicated that severe grades of abruptio placentae are accompanied by escape of thromboplastic proteins into the maternal circulation. This results in varying degrees of defibrination. Platelet participation in this massive coagulation process could be expected to release relatively large quantities of serotonin. Almost all delayed deaths following abruptio placentae are due to renal cortical necrosis of a type which serotonin is capable of producing in the rat.

ELECTRON AND LIGHT MICROSCOPIC STUDY OF THE PATHOLOGIC COURSE OF EXPERIMENTAL RENAL DISEASE IN RATS. *Carolyn F. Piel,* F. W. S. Modern, L. Dong, J. Goodman and R. Moore.* Stanford Univ. School Medicine, San Francisco, Calif., and V. A. Hospital, Long Beach, Calif.

The nephrotoxic serum, prepared by sensitizing rabbits to rat kidney, produces in rats a disease of immediate onset characterized by edema, proteinuria, hypoproteinemia and hyperlipemia.

Weekly autopsied material was prepared by vital osmic fixation for electron microscopic study. Comparable material was formalin-fixed and stained with special mucopolysaccharide and routine stains. Within six hours the glomerular capillary lumina become filled with polymorphonuclear leukocytes. By three days clumps of mucopolysaccharide material are seen in the glomerular capillary lumina which by seven days seem to have become incorporated into the capillary wall. By electron microscopy this thickening of the basement membrane is observed to be a tremendous widening of the lamina densa, the middle layer of the three discernible layers of the basement membrane. Approximately half of the material has fibrin thrombi within the capillary loops in the first two weeks of the disease. Exudation into the capsular space complicates most of the glomerular capillary pathologic changes. Healing of this process results in scarring of the glomeruli.

STUDIES OF THE MECHANISM OF EXPERIMENTAL NEPHROTIC HYPERCHOLESTEREMIA. *Ray H. Rosenman,* Meyer Friedman,* and Sanford O. Byers.** Harold Brunn Inst., Mount Zion Hospital, San Francisco, Calif.

The syndrome induced in rats by injection of rabbit anti-rat kidney serum closely mimics the nephrotic state occurring in humans. It has been found that experimental nephrotic hypercholesteremia, although initiated by the interaction of specific rabbit anti-rat kidney serum with the rat's kidney, is not due to failure of renal excretion of any plasma constituent. It has also been demonstrated that nephrotic hypercholesteremia is an isolated phenomenon confined to the plasma with no evidence of a total error in the body's cholesterol metabolism. Although found to be of endogenous origin, this isolated rise of cholesterol in the plasma was not found to be due to any abnormality in the rat's usual modes of (1) absorbing, (2) excreting, (3) synthesizing cholesterol or (4) to the

* Asterisks indicate members.

ability of the liver of the nephrotic rat to convert and otherwise degrade cholesterol. It is concluded that the isolated accumulation of cholesterol in nephrotic plasma is due to a "trapping" phenomenon which results in retention of cholesterol in the blood and failure of its normal egress from blood to the liver. The behavior of the total lipids parallels that of cholesterol. Other studies will be presented which suggest that the retention of lipids in the plasma may be due to the occurrence of chylomicronemia secondary to abnormal loss, neutralization or utilization of heparin or its clearing process cofactors.

LIPOPROTEIN SEPARATION BY PAPER ELECTROPHORESIS. *John E. Peterson,* Albert L. Chaney,* and Fung-Haan Hsu.* Dept. Internal Medicine and Pathology, College Medical Evangelists, Los Angeles, Calif.

A number of technics for measuring the separation of serum lipoproteins by electrophoretic means have been described. A paper medium is quite convenient and has been used throughout this study. Minor changes in temperature, humidity, buffer strength, voltage and paper are shown to modify results significantly. After electrophoretic separation, the paper strips can be colored by a number of dyes. Both protein and lipoprotein patterns are developed with appropriate staining, and relative changes can be measured by elution or by densitometric scanning. Slight variations in staining, washing and drying of the paper strips will profoundly change the patterns.

With meticulous attention to the variables in electrophoretic separation and staining, good reproducibility can be obtained with a standard error of approximately 5 per cent. With this degree of sensitivity changes in the lipoprotein pattern can be noted following meals of varying fat content, and changes in the gamma, beta and alpha lipoprotein areas can be followed.

PROTEIN METABOLISM IN THE MAMMALIAN KIDNEY. *Alvin L. Sellers,* Harald Eliasch, Jessie Marmorston and Sheldon Rosenfeld.* Inst. Medical Research, Cedars of Lebanon Hospital and the Dept. Medicine, Univ. Southern California School Medicine, Los Angeles, Calif.

It has been adequately demonstrated that plasma proteins pass the glomerular filter and are reabsorbed by the cells lining the proximal convoluted renal tubule. The magnitude of this process has been measured in the rat, employing the dye T-1824. It was estimated that in this species approximately 5 mg. of plasma protein per hour is reabsorbed.

If this material is degraded to amino acids and polypeptides by the tubule cells one might expect to find differences in the concentration of amino acids and polypeptides in renal arterial and venous blood. The concentration of amino acid and polypeptide nitrogen in plasma samples from the aorta, the renal vein and the inferior vena cava of the rabbit has been determined. It is demonstrated that there is a significantly higher concentration of amino acids and polypeptides in the renal vein than in either the aorta or the inferior vena cava. The possible implication of these findings in relation to the role of the kidney in protein metabolism is discussed.

STUDIES IN HUMAN PLASMA PROTEIN FORMATION. II. Fate of Transfused Internally S^{35}-Labeled Plasma Protein and Albumin, Compared with I^{131}-Labeled Albumin, and Plasma Protein Endogenously Synthesized from S^{35}-Labeled Amino Acid Precursors. *Sheldon Margen* and Harold Tarver.* Dept. Physiological Chemistry, Univ. California Medical School, Berkeley, Calif.

In a previous report it was shown that (1) the administration of S^{35} labeled amino acids to study albumin turnover may not be valid because of possible "re-entry" phenomenon from S^{35} amino acids arising from the breakdown of body proteins. Long biological half-lives (thirty-six to fifty-five days) were obtained. Moreover, when this method is employed, "pool size" must be estimated by other technics. (2) The I^{131} technic may be valid but the question of whether the iodine labeled material represents "normal" biologic protein remains unsettled. The apparent half-life of the iodinated albumin changes during the course of the experiment, gradually lengthening.

In order to evaluate the two preceding methods a large dose of S^{35} labeled amino acids was given to a donor. After twenty-four hours a phlebotomy was performed and the plasma separated. In one series of experiments the plasma was administered to normal individuals and the activity of the albumin fraction was determined on specimens withdrawn at intervals. In the other experiments the plasma was fractionated according to the method of Cohn

and the albumin administered to the normal recipients. The results and conclusions obtained from these experiments are as follows: (1) The curves obtained were different from either of the two previous methods. (2) After about six to eight days a single first order exponential curve is obtained with a half-life of twenty-six to thirty-one days. (3) The curves were the same whether plasma or albumin were employed. (4) The pool size can be determined directly with this technic. (5) "Re-entry" is of no consequence with this technic. (6) To date the technic described is the only valid one for measuring albumin "turnover."

INTRA- AND EXTRA-VASCULAR DISTRIBUTION OF CARBON MONOXIDE (CO) AND RADIOACTIVE CHROMIUM (Cr^{51}) IN BLOOD VOLUME DETERMINATIONS. *Reidar Wennesland, Norman Nomof, Ellen Brown* and James Hopper Jr.** Dept. Medicine, Univ. California School Medicine, San Francisco, Calif.

In simultaneous determinations of blood volume with CO and Cr^{51} the values found with CO averaged 16 per cent greater than those obtained with Cr^{51}. Animal experiments were undertaken to demonstrate whether this discrepancy is caused by extravascular accumulation of CO and to determine if possible which tissues have greatest affinity for CO. CO gas and Cr^{51}-tagged erythrocytes were given simultaneously to dogs (4 expts.) and rabbits (11 expts.), and the animals were sacrificed. The ratio CO : Cr^{51} was determined in the blood and used as the unit to which the CO : Cr^{51} ratio of various tissue homogenates was compared. A tissue ratio significantly larger than one was considered indicative of extravascular CO accumulation. Evidence of relative accumulation of CO was found in myocardium and red skeletal muscle. In the dog the average CO : Cr^{51} ratios of these tissues were 3.2 and 4.0 times the blood ratio. Corresponding values for the rabbit were 1.31 and 2.47, probably because of lower contents of myoglobin. A dog which had been perfused with saline showed 7.85 and 32.8.

In contrast, evidence of relative accumulation of Cr^{51} was found in the lung and spleen. In the lungs of dogs and rabbits the average CO : Cr^{51} ratios were 0.85 and 0.65, and in the lungs of the perfused dog 0.34. In rabbit spleen and perfused dog spleen the tissue ratios were 0.77 and 0.06, respectively.

It is concluded that CO accumulates in myocardium and skeletal muscle. Cr^{51} may accumulate in the lung and spleen because the physicochemical properties of erythrocytes are changed by the procedures used.

POTASSIUM CONTENT OF GASTROINTESTINAL SECRETIONS AND ITS RELATION TO TOTAL EXCHANGEABLE POTASSIUM. *N. J. Sweet, J. Nadell and I. S. Edelman.** Dept. Medicine, Univ. California School Medicine, San Francisco, Calif.

The gastrointestinal pool of potassium is not considered in present calculations of shifts of that ion in response to metabolic stimuli. Neither is it considered as a possible homeostatic reservoir when there are abnormal losses of potassium from the body. In this study we have measured the fraction of the total exchangeable potassium contained in the lumen of the gastrointestinal tract. Thirty-eight rabbit experiments have been performed. K^{42-43} was used to measure the distribution of exchangeable body potassium. Following administration of the isotope, samples of blood and urine were obtained and the gastrointestinal tracts, from the cardia of the stomach to the mid-transverse colon, were removed. These animals were studied after fasting periods varying from four to forty-two hours. The minimum period of isotope equilibration was twenty-one hours.

The following results were obtained: (1) Intralumenal gastrointestinal tract potassium content averaged 6.2 per cent of the total exchangeable potassium with a range of 2.6 to 9.8 per cent. (2) The partition of potassium in the gastrointestinal tract expressed as per cent of total exchangeable potassium was (a) stomach 0.6 per cent (range 0.3 to 1.3 per cent), (b) small intestine 1.2 per cent (range 0.5 to 2.3 per cent) and (c) cecum and proximal transverse colon 4.6 per cent (range 1.5 to 7.6 per cent). (3) The specific activity of gastrointestinal contents indicated that radiopotassium exchange is complete at forty hours. (4) In twelve rabbits forty-hour total fecal excretion of potassium averaged 3.3 mEq. (range 0.4 to 9.0 mEq.) and was therefore significant in some instances.

These data indicate that a significant proportion of the total body potassium pool of rabbits is within the lumen of the gastrointestinal tract and that this fraction is an additional subcompartment to be considered in body potassium distribution.

POTASSIUM WASHOUT BY ENEMAS. *Marcelle Dunning and Fred Plum.* Div. Neurology, Dept. Medicine Univ. Washington, Seattle, Wash.

The discovery of profound hypokalemia in a man with a cauda equina lesion led to the hypothesis that excessive potassium washout had occurred during enemas. Blood, urine and fecal contents were analyzed for potassium in this man and in other patients requiring either sporadic or repeated tap water enemas. As much as 250 mEq. of potassium was found in a single enema efflux. Potassium washout varied, however, from patient to patient and was as small as 0.5 mEq. in some instances. Serum potassium fell as much as 1.5 mEq. when washout potassium was large. Immediate urinary conservation failed to compensate bowel potassium loss. When contact between large amounts of enema fluid and bowel wall was prolonged, patients with neurogenic bowel dysfunction had the most potassium in their enema returns. Clinical symptoms of hypopotassemia were seen only when body stores had been depleted. Effective bowel care reduced the fluid and the time required for stool evacuation and prevented significant potassium wastage.

EFFECT OF RESPIRATORY ACIDOSIS ON THE INTERNAL DISTRIBUTION OF POTASSIUM. *B. H. Scribner,* J. M. Burnell and Kenneth Fremont-Smith.* V. A. Hospital and Dept. Medicine, Univ. Washington School Medicine, Seattle, Wash.

Several investigators, including Fenn, Abrams and particularly Keating, have demonstrated that metabolic acidosis causes hyperkaliemia by effecting a transfer of potassium out of the cells while metabolic alkalosis leads to hypokaliemia and an inward movement of potassium. Contrariwise the muscle analysis data of Darrow have been interpreted by many to indicate that acid-base imbalance has just the opposite effect on the internal distribution of potassium.

In this study respiratory acidosis was produced by allowing ten dogs to breathe 30 per cent CO_2 and 70 per cent oxygen, the pH falling to about 6.9. Ten additional dogs served as controls. Five dogs in each group had ureteral ligation just prior to the study. There was no significant change in the serum potassium level of anesthetized controls but in dogs with respiratory acidosis there was a gradual rise in serum potassium to levels of 6 to 9 mEq./L. Internal balance calculations using the chloride space demonstrated that this rise in serum potassium was due to transfer of potassium out of the cells.

Potassium loads were given intravenously to five normal dogs and to five dogs with respiratory acidosis. Eighty per cent of the potassium retained by the normal dogs entered the cells. None of the potassium retained by the acidotic dogs entered the cells.

These results demonstrate that respiratory acidosis causes a transfer of potassium out of the cells. This is similar to the transfer observed in metabolic acidosis by Keating.

STUDIES ON THE MECHANISM OF RENAL TUBULAR ACIDOSIS. *Telfer B. Reynolds and Helen E. Martin.** Dept. Medicine, Univ. Southern California School Medicine and the Los Angeles County Hospital, Los Angeles, Calif.

Three adult patients with well documented renal tubular acidosis have been studied in an effort to elucidate the mechanism responsible for the syndrome. All demonstrated low basal urinary ammonia and titratable acid excretion and a relatively constant urine pH in the vicinity of 6.5. Infusion of 10 cc./kg. of isotonic neutral phosphate solution resulted in a 15-fold rise in urinary phosphate excretion, a mean increase in titratable acidity from 16 to 130 μEq./minute and a mean drop in pH from 6.67 to 6.39. Infusion of 7 per cent $NaHCO_3$, 5 cc./kg., resulted in a rise in serum bicarbonate levels from a mean of 17.5 to 28.3 mEq./L. and an increase in tubular bicarbonate reabsorbtion from 1.7 to 2.8 mEq./minute/100 cc. glomerular filtrate. Bicarbonate excretion did not change significantly (23 μEq./minute).

In two of the patients oral administration of 2.0 mEq./kg./day of ammonium chloride produced a progressive symptomatic acidosis without appreciable increase in urinary ammonia or titratable acid excretion. However, equivalent oral doses of acid phosphate salt were well tolerated, acidosis did not progress and urinary titratable acidity increased fourfold.

The carbonic anhydrase system, as suggested by others, appeared capable of marked increase in activity under certain circumstances. Although bicarbonate excretion is increased in the basal state appropriate to the relatively high urine pH, tubular reabsorptive capacity for bicarbonate is not impaired. A method of increasing the low basal ammonium excretion has not been found. Current etiologic theories do not seem to be consistent with these data.

ROLE OF LACTIC ACID IN METABOLISM. *Douglas R. Drury* and Arne N. Wick.** Dept. Physiology, School Medicine, Univ. Southern California, Los Angeles, and Scripps Metabolic Clinic, La Jolla, Calif.

Lactic acid is ordinarily considered not as a fuel but as a breakdown product of anaerobic glycolysis that is reconverted to glucose by the liver before it is oxidized. Workers in the past have doubted that lactic acid can be oxidized directly by the muscles, although according to one view the muscles can convert it to glycogen. A study has been made of this problem in the eviscerated animal, making use of radioactive labeled lactate. It has been found that lactate is rapidly and preferentially burned to carbon dioxide by the extrahepatic tissues. In this preparation there is a high turnover rate of lactate. These results indicate that there is also a high turnover rate in the intact animal, although the concentration of lactic acid is kept low by its oxidation by the peripheral tissues and its conversion to glucose by the liver.

METABOLIC PATHWAYS OF COPPER METABOLISM. *J. P. Mahoney, J. A. Bush, C. J. Gubler, G. E. Cartwright,* and M. M. Wintrobe.** Dept. Medicine, Univ. of Utah, College Medicine, Salt Lake City, Utah.

Following the intravenous administration of radioactive copper to normal dogs, 0.6 per cent of the injected dose was recovered in the urine and 11.7 per cent in the stools during a five-day period. In dogs with the bile duct ligated, 2.8 per cent of the radioactive copper was recovered in the urine and 3.2 per cent in the stool. In dogs with the bile duct transposed to the urinary bladder, 12.3 per cent of the injected dose was recovered in the urine plus bile and 1.5 per cent in the stools. Similar observations have been made in normal human subjects except that a smaller fraction of the injected dose has been recovered in the excreta.

Following the intravenous injection of radioactive copper into human subjects, radioactivity in the plasma decreased rapidly and reached a low level within two hours. Thereafter, during the next four days there was a progressive rise in the radioactivity of the plasma.

VALUE OF GONADOTROPIN ASSAYS IN THE DIFFERENTIAL DIAGNOSIS OF MYXEDEMA. *Paul P. VanArsdel, Jr. and Robert H. Williams.** Dept. Medicine, Univ. Washington School Medicine, Seattle, Wash.

The differentiation of Simmonds' disease from primary hypothyroidism poses problems. We have studied the findings in ninety-four adult patients, who have shown documented hypothyroidism in the presence or absence of pituitary deficiency. There were thirty-five cases of primary myxedema; of the patients with Simmonds' disease, nineteen were due to a chromophobe adenoma, twelve to postpartum necrosis of the pituitary, three to a craniopharyngioma, two to an eosinophilic adenoma, one to an aneurysm of the internal carotid artery and twenty-two to pituitary failure of unknown cause. Special attention was paid to serum cholesterol levels, I^{131} response to thyroid-stimulating hormone, and urinary gonadotropin assays. In our experience adrenal response to ACTH and insulin tolerance tests were not of as much value in differentiating primary from secondary myxedema.

The serum cholesterol was significantly elevated in 71 per cent of the patients with primary myxedema but was elevated in only 13 per cent of those with secondary myxedema despite good nutritional status in a majority of the latter. As has been reported by others, thyroid I^{131} uptake averaged higher than in primary myxedema, although protein-bound iodine levels in serum were not significantly different in our two groups. In patients with secondary myxedema, thyroid-stimulating hormone, given intramuscularly 10 units per day for three days, produced elevations of I^{131} uptake to levels ranging from 21 per cent to 75 per cent without any particular correlation with the severity of the clinical picture. The urinary gonadotropin levels were of special diagnostic value in the postmenopausal females who comprised 83 per cent of our group of patients with primary myxedema and 36 per cent of those patients with pituitary deficiency not due to postpartum necrosis. Urinary gonadotropins were not present in any of thirty-seven patients with pituitary deficiency. In twenty postmenopausal women with primary myxedema, gonadotropins were found at the usual high amounts in sixteen, were present only at low levels in two and were absent in two. Because the occasional patient with pituitary myxedema may have a thyroid gland unresponsive to thyroid stimulating hormone, additional diagnostic tests, especially the gonadotropin assay, are indicated.

ASSAY OF THYROTROPIN (TSH) USING THYROID TISSUE SLICES. *John L. Bakke* and Nancy Lawrence.* Dept. Medicine, V. A. Hospital, Univ. Washington, Seattle, Wash.

Surviving beef thyroid slices were incubated for one hour in a modified Krebs' medium labeled with radio-iodide and then transferred to non-radioactive medium to measure the release of radio-iodide from the slice into the medium. Propylthiouracil, 10^{-3} M, was present in all mediums to prevent organic binding, thereby limiting the study to inorganic radio-iodide. TSH added *in vitro* to the release medium caused the slice to retain twice as much radio-iodide as did controls. Expressed as a tissue/medium iodide concentration ratio (T/M) a control level of 85.4 ± 9.67 (mean ± standard error) was increased to 147.7 ± 18.2 by 0.08 U.S.P. milliunits of TSH ($t_{61} = 17$, highly significant). The T/M response was proportional to the log-dose of TSH between 0.001 and 0.1 m.u., a range believed to include the normal human serum level of TSH.

Using a model consisting of two compartments, the medium iodide space and the tissue iodide space, the measured release of tissue radio-iodide over a four hour period permitted one to solve for the rate constants k_1, representing the release of iodide from the slice into the medium, and k_2, representing the transport of iodide back into the slice. In a preliminary experiment the control value of k_1 was 0.336 and k_2 was 0.378. TSH increased k_1 to 0.482 and k_2 to 0.820. Although TSH increased both rates, it increased uptake more than release and increased iodide flux ($k_2 - k_1$) eightfold as compared with controls.

These experiments support the hypothesis that TSH directly stimulates the iodide-collecting mechanism providing a sensitive response to TSH which may be directly applicable to human serum.

OBSERVATIONS OF SODIUM-RETAINING CORTICOID (ALDOSTERONE) IN HUMAN URINE. *Robert H. Curtis, Bernard J. Axelrad, Ben B. Johnson, and John A. Luetscher, Jr.** Dept. Medicine, Stanford Univ. School Medicine, San Francisco, Calif.

Sodium-retaining corticoid has been found in the urine of normal men, and increased amounts have been recovered from the urine of patients with reduced sodium excretion. The sodium-retaining corticoid from the urine of a patient with nephrosis has been identified as aldosterone by Neher and Wettstein. Aldosterone has not been found in the urine of patients with Addison's disease or in those subjected to bilateral adrenalectomy. The output of this hormone is apparently independent of pituitary control since a normal quantity of aldosterone is found in the urine of patients with panhypopituitarism and in those subjected to hypophysectomy. Marked restriction of sodium intake is followed by increased output of aldosterone. The basis for increased aldosterone output in patients with nephrotic syndrome, congestive heart failure, Laennec's cirrhosis or toxemia of pregnancy is being studied.

In general, increased aldosterone output is accompanied by reduced sodium excretion. In polycystic disease of the kidneys and in congenital adrenal hyperplasia, a normal or increased output of aldosterone may be accompanied by urinary sodium loss, presumably because the effectiveness of aldosterone is reduced.

CLINICAL STUDIES WITH 9-ALPHA-FLUOROHYDROCORTISONE. *R. H. Orr, V. Di Raimondo, M. E. Flanagan, and P. H. Forsham.** Metabolic Unit Research in Arthritis and Allied Diseases and Dept. Medicine, Univ. California School of Medicine, San Francisco, Calif.

In the rat and dog 9-alpha-fluorohydrocortisone (FF) has been shown by others to be approximately twenty times as potent as hydrocortisone in glucocorticoid and eosinopenic activity and three to five times as potent as desoxycorticosterone in electrolyte regulation. Potencies of the same order of magnitude have been observed in the experiments with human subjects to be presented. Balance studies in a bilaterally adrenalectomized-oophorectomized subject indicate that FF is considerably more than ten times as active as hydrocortisone in producing sodium retention. This effect is the limiting factor in systemic use of this compound in therapy. Topically applied 0.25 per cent FF acetate ointment appears more effective than 2.5 per cent hydrocortisone acetate in the treatment of certain inflammatory and allergic skin conditions. In such topical uses its advantage in superior anti-inflammatory potency may be explicited without undesirable side effects.

FF has been found to be a highly effective suppressant of adrenal cortical secretion. Since the metabolites of FF contribute negligibly to urinary 17-keto- and 17-hydroxy-steroids, the

measurement of these hormones accurately reflects adrenal function. Five to 10 mg. of FF given as an eight hour intravenous drip, or 1 to 2 mg. by mouth every six hours, markedly suppress adrenal cortical output. Studies of the pituitary-adrenal relation in normal as well as abnormal states of adrenal cortical function will be presented.

Preliminary results from a study of urinary metabolites of FF indicate that degradation and conjugation are similar to that of hydrocortisone. These findings, in conjunction with the observed high topical potency, support the conclusion that the potentiality of action affected by halogen substitution probably occurs at the cellular level in the target tissues.

CIRCULATING CONCENTRATIONS OF ADRENOCORTICOTROPHIN IN RHEUMATIC FEVER. *A. Bertrand Brill, Robert S. Ely* and Vincent C. Kelley.** Univ. Utah School Medicine, Salt Lake City, Utah.

A satisfactory determination of endogenous ACTH levels in blood has become possible only recently. Development of the adrenal ascorbic acid depletion method for assaying the ACTH content of blood has been applied in investigations with animals and more recently in clinical investigations in occasional patients. This method allows assessment of pituitary function permitting a separate analysis of the factors involved in the pituitary-adrenal glands. In the present study endogenous ACTH activity was determined in the blood of forty children. These included normal control groups, subjects in various stages of rheumatic fever and its therapy and patients with Sydenham's chorea. In the group of normal children, ACTH concentrations were not detectable. In rheumatic fever patients, before administration of hormone therapy, high levels of circulating ACTH were found which varied with the acuteness of the disease. Subsequently these levels were influenced by the type of therapy administered.

ERYTHROCYTE CARBOHYDRATE METABOLISM IN HEREDITARY HEMOLYTIC ANEMIAS. *A. G. Motulsky, B. W. Gabrio,* J. Burkhardt and C. A. Finch.** Dept. Medicine, Univ. Washington, Seattle, Wash.

Most hereditary hemolytic anemias are characterized by non-specific morphologic red cell anomalies. Abnormal shape may be absent altogether, as in hereditary non-spherocytic hemolytic disease. Structural changes fail to explain increased destruction in most instances. Since glucose is the prime source of energy of the red cell, an investigation of carbohydrate metabolism of abnormal erythrocytes appeared desirable. Methods included measurements of erythrocyte glycolytic rates, P^{32} uptake and analysis of non-radioactive and tagged phosphorylated intermediates by differential hydrolysis and by paper electrophoresis and chromatography. Radioautographs of paper strips allowed ready screening, visualization and densitometric quantitation of metabolic abnormalities.

Total P^{32} uptake and glycolytic rates of mature cells were normal in hereditary non-spherocytic hemolytic disease and in hereditary spherocytosis as well as in all other hemolytic anemias studied. Disturbed intracellular distribution of phosphorylated intermediates was detected in several cases of hereditary non-spherocytic disease and in hereditary spherocytosis. In the former condition, increases of 2,3-diphosphoglycerate were found, while the defect in hereditary spherocytosis consisted of accumulation of intraerythrocytic inorganic phosphate during incubation with P^{32}. Glucose and adenosine *in vitro* corrected the latter abnormality. The abnormal pattern showed little intrafamilial variability but differed from family to family.

Incubation of hereditary spherocytes for twenty-four hours at 37°C. causes marked increases in osmotic fragility. Such a metabolic stress test demonstrated the biochemical defect in another manner since glucose, adenosine and related nucleosides tended to correct the abnormal fragility.

These studies demonstrate the existence of apparently genetically controlled metabolic lesions in abnormal red cells. It has been shown that a reversible erythrocytic defect produced by red cell storage seriously interferes with viability of normal stored cells. It is suggested that the demonstrated lesions in hereditary hemolytic anemias may similarly be significant in producing the shortened red cell life span of abnormal cells.

DESTRUCTION OF BOVINE ANTIHEMOPHILIC FACTOR BY A COMPONENT OF HUMAN PLASMA. *Theodore H. Spaet* and Evelyn J. Garner.* Stanford Univ. School Medicine, San Francisco, Calif.

Although the antihemophilic factor (AHF) of human plasma is storage-labile, bovine AHF is

stable even after prolonged periods of storage. The difference between human and bovine AHF lability could be due to intrinsic differences in the protein molecule, a destructive factor in human plasma, or a protective factor in bovine plasma. To determine which of these hypotheses was more likely, AHF assays were done on mixtures of human and bovine plasma which had been incubated together at room and refrigerator temperatures. Control assays were undertaken on these same plasmas which had been incubated separately and mixed just prior to testing. Additional control assays were done on mixtures of plasmas incubated with saline. AHF was measured both by the thromboplastin generation test and by the ability of a specimen to improve the prothrombin consumption of hemophilic blood.

The AHF content of bovine plasma incubated with human plasma was lower than that of bovine plasma incubated with saline. Fresh mixtures of bovine and human plasma which had been incubated separately were as active or more active than bovine plasma incubated with saline. These data indicate a component in human plasma which destroys bovine AHF. Additional studies were made on the properties of this component. It appeared to be in fraction III of Cohn, was not destroyed by heating to 56°C., was not adsorbed on barium sulfate and was less active in serum than in plasma. Plasma from patients with hemophilia or with a circulating anticoagulant did not have greater than normal destructive activity. The data suggest that human AHF is unstable because of a plasma component which destroys or inactivates the protein.

CAPACITY FOR LEUKOCYTE AND PLATELET PRODUCTION AS STUDIED BY LEUKOPHERESIS. *C. G. Craddock, Jr.,* S. Perry and J. S. Lawrence.** Univ. California School Medicine, Los Angeles, Calif.

A technic of rapid mechanical removal of leukocytes and platelets from peripheral blood, termed leukopheresis, has been applied to dogs. Clearance of these elements at a rate in excess of one blood volume per hour for two to three hours results in marked leukopenia and thrombopenia. The reappearance of cells follows a fairly uniform pattern. For one to two hours after a single sustained leukopheresis the white cell level remains low. Steady, linear rise then begins, reaching baseline in four to five hours and continuing to 200 per cent to 400 per cent above baseline where it remains for two to three days, slowly declining to normal.

Repeated leukophereses on successive days indicate that the normal animal can rapidly replenish the peripheral blood with leukocytes even though several times the number in the peripheral blood are removed. Following three days of leukopheresis the white blood cell level rises to a peak of 30,000 to 50,000/mm.^{3} on the fifth to sixth day. The marrow changes indicate myeloid hyperplasia and immaturity beginning on the third to fourth day becoming extreme on the fifth day. Dogs exposed to heavy irradiation one hour prior to leukopheresis can still develop a leukocytotic response, although to a limited degree. This response becomes progressively less as the marrow decreases in cells.

Thrombocytopenia induced by rapid clearance of these elements from the peripheral blood persists for three to four days, with megakaryocytic hyperplasia.

IN VIVO LIFE SPAN OF LEUKOCYTES OF LEUKEMIC PATIENTS AS MEASURED BY RATE OF INCORPORATION OF P^{32} IN DNA FRACTION. *Demetrios A. Rigas and Edwin E. Osgood.** Div. Experimental Medicine, Univ. Oregon Medical School, Portland, Ore.

P^{32} is incorporated into DNA only when a cell divides. The rate of this incorporation may be used to determine the life span and the intermitotic interval of the cells. Ten patients with chronic granulocytic, eight with chronic lymphocytic, two with acute lymphocytic and three with subacute monocytic leukemia were given intravenous injections of inorganic P^{32} and daily booster injections to keep the plasma level constant. Daily blood samples were obtained, the leukocytes were separated by the phytohemagglutinin technic, the DNA was isolated by the Schmidt-Thannhauser method and its phosphorus and P^{32} determined. The DNA specific activity expressed as the per cent of that of the plasma was plotted against time and the curves were analyzed with the aid of equations derived theoretically. With adequate data these equations yield the life span and the intermitotic interval separately. However, from our data only their sum could be derived. This was found to be 5.08 $\pm$ 1.12 days for the chronic granulocytic, 4.07 $\pm$ 0.41 days for the subacute monocytic, 4.35 $\pm$ 1.46 days for the acute lymphocytic, and, in high contrast, 84.2 $\pm$ 32.0

days for the chronic lymphocytic leukemias. An analysis of variance disclosed no significant difference at the 5 per cent level among the first three but the difference in the chronic lymphocytic is obviously highly significant.

It is interesting that the figure of 5.08 ± 1.12 days for chronic granulocytic leukemia is not significantly different from that of 106 hours derived by Osgood from marrow culture data.

COMPARISON OF RELATIVE RATES OF FORMATION OF NEW LEUKOCYTES IN MYLERAN TREATED AND UNTREATED PATIENTS WITH CHRONIC GRANULOCYTIC LEUKEMIA: Measured by Uptake of Radioactive Phosphorus in Isolated Desoxyribonucleic Acid. *Jonah G. Li,* M. E. Leonard and Gerald Harrison.* San Francisco, Calif.

A comparison has been made between the relative rates of formation of new leukocytes in patients with untreated chronic granulocytic leukemia and in patients with the identical disease but treated with myleran (1,4-dimethylsulfonyloxybutane). The Osgood technic (Cancer, 5: 331, 1952) of measuring the uptake of radioactive phosphorus (P^{32}) in the isolated desoxyribonucleic acid (DNA) was utilized as an index of the relative rates of formation of new leukocytes.

It was found that myleran, given to four patients with chronic granulocytic leukemia in a dose of 100 mg. divided over a four-day period, suppressed the P^{32} uptake by the leukocyte DNA to approximately 20 per cent of that obtained in the untreated patient. In a single patient with chronic granulocytic leukemia treated with a lower dosage of myleran, 2 and 4 mg. on alternate days continuously, the P^{32} uptake by the leukocyte DNA was reduced to approximately 35 per cent of that of the untreated level. These findings are interpreted as indicating that myleran is effective in lowering the number of circulating leukocytes by virtue of a cytostatic action (inhibition of mitosis). It is felt that if its action were primarily cytotoxic there would be essentially no suppression of P^{32} uptake by the DNA.

CLINICAL EVALUATION OF ADMINISTRATION OF 6-MERCAPTOPURINE, 6-THIOGUANINE AND AZASERINE IN ACUTE AND CHRONIC LEUKEMIA. *B. E. Hall,* F. M. Willett, E. B. Reed, W. F. Dowling and T. V. Feichtmeir.* San Francisco, Calif.

During the past eighteen months thirty-nine patients with acute leukemia, six patients with chronic granulocytic leukemia and one patient with chronic lymphocytic leukemia have been treated with 6-mercaptopurine alone or in combination with 6-thioguanine or azaserine. Of the thirty-nine patients with acute leukemia (five children and thirty-four adults) eight received an inadequate trial of therapy. Of the thirty-one patients receiving adequate therapy, remission was induced initially in nineteen patients (61 per cent). In four adults remission was complete, lasting fifteen, twelve, six and three months, respectively. No treatment was given during complete remission. Two of the four patients are still living and well; two relapsed and died.

Of fifteen patients in whom partial remission was induced, maintenance therapy frequently was utilized. Five patients became resistant to the administration of 6-mercaptopurine alone. In two, remission was obtained by combination therapy (6-mercaptopurine and azaserine); three failed to respond to the simultaneous administration of 6-mercaptopurine and azaserine or 6-mercaptopurine and 6-thioquanine.

Details of dosage schedules, survival times, complications of treatment and biochemical studies relating to urinary excretion of uric acid, amino nitrogen, phosphorus, and so forth in acute leukemia will be presented. The results of treatment in seven patients with chronic leukemia was discussed.

BERNHEIM'S SYNDROME: IS IT A CLINICAL ENTITY? *Arthur Selzer,* Herbert W. Bradley and Forrest M. Willett.* Medical Service, V. A. Hospital and Dept. Medicine, Stanford Univ. School Medicine, San Francisco, Calif.

In the past decade there has been a revival of interest in the concept of isolated right ventricular failure appearing in the course of left-sided cardiac lesions thought to be due to deviation of the cardiac septum to the right resulting in "right ventricular stenosis" (Bernheim, 1910).

A study of circulatory dynamics was made of a patient who fulfilled all the clinical criteria for "Bernheim's syndrome," the postulated findings of which were confirmed at autopsy. It was found that the patient had marked elevation of left atrial pressure and pulmonary hypertension in addition to signs of right ventricular failure. These findings are inconsistent with the traditional concept of Bernheim's syndrome for they point to the conventional chain of events of left ventricular failure—pulmonary hypertension—right ventricular failure in spite of the clinical

absence of dyspnea and signs of pulmonary congestion. A further scrutiny of the Bernheim concept revealed that its pathologic criterion is based on a misconception, because a "deviation" of the septum encroaching on the cavity of the right ventricle can be demonstrated in all cases of left ventricular hypertrophy, regardless of the clinical pattern, and is frequently present in normal hearts. It is concluded that "Bernheim's syndrome" is not a clinical entity but merely represents a variant of conventional cardiac failure in left ventricular lesions in which the stage of left ventricular failure is clinically inconspicuous.

EARLY SYSTOLIC CLICK OF THE PULMONARY ARTERY. *Herbert N. Hultgren and John J. Kelly, Jr.* Dept. Medicine, Stanford Univ. School Medicine and State Univ. New York College Medicine, New York, N. Y.

In occasional patients with pulmonary hypertension a sharp sound closely following upon the apical first sound is present at the pulmonic area. This has been termed the semilunar opening click by Wolferth although it may not be of valvular origin. Twenty-five patients exhibiting such early systolic clicks have been studied clinically and by phonocardiography to investigate further the characteristics and mode of production of this sound. In addition cardiac catheterization and electrokymographic studies have been made to relate the time of occurrence of the click to the mechanical events in the right portion of the heart.

All patients studied had severe pulmonary hypertension, and in eight cases this was associated with an atrial septal defect. The click was maximal over the pulmonic area in the majority of cases, and it occurred from .04 to .09 seconds (mean .07 seconds) after the apical first sound. At the third interspace along the left sternal border it often resembled a split first sound but the second component was loud over the pulmonic area and faint or absent at the apex. The first sound-click time is related to the duration of the isometric contraction time of the right ventricle. The click occurs simultaneously or a brief instant after the rise of pressure in the pulmonary artery at the time when the wall of the pulmonary artery is being most rapidly tensed. These observations are in accord with those reported by Leatham and Vogelpoel in 1953.

When the sound is present and is widely separated from the first sound it is a reliable indication of severe pulmonary hypertension.

DISCREPANCIES BETWEEN SUBJECTIVE AND OBJECTIVE EVALUATION OF MITRAL COMMISSUROTOMY. *Gordon G. Bergy and Robert A. Bruce.** Univ. Washington, Seattle, Wash.

Clinical observation has revealed limited and gradual improvement as well as recurrent complications in many patients submitted to mitral commissurotomy. This experience is contrary to some of the enthusiastic reports of surgical treatment of mitral stenosis. In addition, some patients report improvement in symptoms which is not confirmed by disappearance of signs of rheumatic heart disease and its complications. In order to evaluate this confusing situation a group of thirty unselected patients have been restudied from four to thirty-eight (mean twenty-one) months after surgery. Histories were reviewed with respect to symptoms, present capacity for work and recreation, limitations of physical activity including need for daily rest periods and drugs used in continued medical treatment. Physical examination was supplemented by careful chest fluoroscopy, electrocardiogram, vital capacity and circulation time. Tolerance for a standard exercise test was determined and compared with serial preoperative and postoperative tests.

Twenty-nine patients claimed marked subjective improvement. Three were unable to work, three were working part time and three had easier jobs than before surgery. Many who claimed to be entirely well were not able to meet the requirements of ordinary daily life. Whereas five participated in sports, one-third were unable to participate in any recreational activity. Four showed signs of frank congestive heart failure when re-examined. Only thirteen patients had normal exercise tolerance. Subnormal performance was usually due to impaired respiratory efficiency (associated with low vital capacity and 0.5 second expiratory volume) and tachycardia. Four patients exhibited impaired endurance.

It is concluded that there are appreciable discrepancies between subjective and objective evaluation of mitral commissurotomy. Much of limited improvement by objective criteria relates to subnormal pulmonary function.

THE PATH OF EXCITATION IN CLINICAL AURICULAR FLUTTER. *David L. Bruns and David A. Rytand.** Dept. Medicine, Stanford Univ. School Medicine, San Francisco, Calif.

Circus movement as a hypothetical mechanism responsible for human auricular flutter seemed to be supported by the observation that, with this arrhythmia and without quinidine, the auricular rate is distinctly slower than usual in certain patients with great auricular enlargement.

Exploration of the excitation wave in two such cases by means of electrodes in the esophagus and on the anterior portion of the chest wall suggests that the long auricular cycle is related to increased path length rather than to slow velocity. The wave appeared to ascend posteriorly and to descend anteriorly as if returning. Apparent intrinsicoid deflections were recorded near the xiphoid as late as 50–75 per cent of the cycle length, timed by waves low in the esophagus within the phase alleged to represent repolarization. In one patient with a cycle length of 0.33 seconds the wave was found to be primarily negative at the manubrium (0.15 seconds) as well as at the lower esophagus (0.00 seconds) and from configuration alone might have commenced near either site; it was positive near the xiphoid (0.25 seconds). In the second patient the cycle length was 0.34 seconds; the wave was negative low in the esophagus (0.00 seconds) but positive at the manubrium (0.12 seconds) as well as at the xiphoid (0.18 seconds).

In a patient with normal auricular size and flutter at a more usual rate (cycle 0.25 seconds) the wave also was found to be negative low in the esophagus (0.00 seconds), positive at manubrium (0.05 seconds) and xiphoid (0.12 seconds). Similar observations suggesting circus movement have been reported by others.

HYPOPHYSECTOMY IN PATIENTS WITH DIABETIC VASCULAR DISEASE; CLINICAL AND METABOLIC OBSERVATIONS. *Laurance W. Kinsell,* Lester Lawrence and Robert D. Weyand.* Dept. Medicine and Neurosurgery, Samuel Merritt Hospital and Inst. for Metabolic Research of Highland Alameda County Hospital, Oakland, Calif.

In these institutions more than a year has elapsed since the first hypophysectomy was performed in a patient with malignant diabetic vascular disease. On the basis of experimental observations and observations in patients with Sheehan's syndrome superimposed upon diabetes mellitus, it was predicted prior to surgery that hypophysectomy would transform severe insulin-resistant diabetes to mild insulin-sensitive diabetes and would result in significant decrease in hypertension. It was hoped that by removal of the pituitary and adrenocortical hormones and amelioration of the diabetes, progression of the vascular disease would be slowed or stopped and that possibly improvement in lesions which were still potentially reversible might occur.

Five patients have been hypophysectomized. One of these has been re-operated upon because of a less than total hypophysectomy at the time of the first operation. There has been no operative mortality. The first two patients had extensive renal damage and died three and one-half and four and one-half months after surgery, respectively, essentially as the result of renal insufficiency. All patients have had striking increase in insulin sensitivity and disappearance of hypertension when the latter was present.

In the surviving patients, on the basis of evaluation of the optic fundi, of renal function and of albuminuria, there appears to be good reason to believe that progression of the pathologic vascular condition has been halted. Evidence strongly suggests improvement in some of the existing pathologic vascular conditions.

It is of interest that total adrenalectomy done in similar patients (personal communications from Halmi and from Conn) fails to cause any major decrease in insulin requirement. This suggests that the non-ACTH pituitary "diabetogenic factor" is chiefly responsible for the relative insulin resistance in these patients.

CLEARANCE OF RADIOACTIVE IODINE FROM THE EXTREMITIES IN PATIENTS WITH MYOCARDIAL INFARCTION. *F. K. Bauer,* F. R. Mugler, Jr., V. M. Lieberman and J. P. Westergart.* Radioisotope Unit and Medical Service, Wadsworth Gen. Hosp., V. A. Center, and Dept. Medicine, Univ. California Medical Center, Los Angeles, Calif.

It has been previously shown that tracer amounts of I^{131}, Na^{24} and K^{42} in small amounts of normal saline solution can be used interchangeably in clearance tests from injection sites. It has also been shown that clearance determinations after needle injection do not give reproducible results but that jet injection does. Tracer amounts of one microcurie I^{131} in 1 ml. sterile normal saline were injected by hypospray into the thighs and arms of healthy control subjects and of thirty-five patients with myocardial infarction. All subjects were in the supine position. The solution penetrates into the muscle. The disappearance of the injected substance is

measured with a scintillation counter which is held over the injection site. The time required for half of the radioactivity to disappear from the injection site in normal controls is from seven to fourteen minutes, with a mean of nine minutes.

It is believed that the local isotope clearance is an index of the state of the capillary bed. Two different observations are presented in support of this belief: (1) A patient with coarctation of the aorta with absent pulsations in the lower extremities had a normal clearance time in the arms and a threefold prolongation of the clearance time in the legs. (2) Two healthy control subjects received intravenous infusions of norepinephrine which more than doubled their clearance times.

None of the thirty-five patients with myocardial infarction had clinical signs and symptoms of congestive failure. The venous pressures were within normal limits as were their circulation times (Decholin). Greatly prolonged clearance times were found in these patients for several weeks and even months following myocardial infarction. Some patients received norepinephrine (Levophed) early in the study; their peripheral tissue clearances were delayed.

After the peripheral clearance in the supine position had returned to normal it was found, when some of the patients were placed in the sitting position, that the clearance times again became greatly delayed. This is not found in normal controls. This phenomenon of postural change is present both in the legs and arms but not invariably in the same patient.

It is believed that the delayed clearance of peripherally injected I^{131} in patients with myocardial infarction is caused by a diminution of the peripheral capillary bed. Whether this phenomenon is mediated through a reflex or a humoral mechanism is now under investigation.

MAXIMUM EXPIRATORY FLOW; A BEDSIDE PULMONARY FUNCTION TEST. *John R. Goldsmith.* Dept. Medicine, V. A. Hospital, Univ. Washington, Seattle, Wash.

The maximum breathing capacity test (MBC) has become accepted for measuring ventilatory function. Being arduous for the patient, and technically difficult to perform, the MBC test has been supplanted in some laboratories by timed vital capacity tests. All such tests depend on the maximum flow rate of air during expiration. Measurement of this rate has been undertaken, and its significance as a measure of ventilatory capacity is assessed. A simple, rugged, indicating flowmeter has been designed with a linear response to flow rates up to 200 liters per minute (± 3.9 L./minute). It has been used in tests on normal subjects and in patients with various types of pulmonary insufficiency. Even in advanced disease a reliable measurement may be made by taking a sufficient number of observations. The validity of tests with this portable instrument has been compared with simultaneous measurements on a sensitive, high speed, recording flowmeter during the maximum expiratory flow maneuver. The relationship of determinations by these two methods is discussed.

The correlation between maximum expiratory flow and maximum breathing capacity in forty-four patients with varying degrees and types of pulmonary disease is shown to be greater than $+.79$, confirming the validity of the maximum expiratory flow as a test of the obstructive component in pulmonary insufficiency. Its performance is as simple as a measurement of vital capacity and as easy to reproduce. Together with the vital capacity it provides a comprehensive description of ventilatory capacity.

PREGNANCY: A FACTOR RESPONSIBLE FOR BIOLOGIC FALSE POSITIVE REACTIONS AS DETERMINED BY THE TREPONEMA PALLIDUM IMMOBILIZATION TEST. *Charles M. Carpenter,* Ruth A. Boak, James N. Miller, Helene E. Drusch, John M. Chapman and Gerald A. Heidbreder.* Dept. Infectious Diseases, School of Medicine, Univ. California, Los Angeles City and Los Angeles County Health Dept., Los Angeles, Calif.

Positive standard serologic tests for syphilis (STS) in the absence of clinical evidence of the disease presents a difficult diagnostic problem. With the development of the Treponema pallidum immobilization (TPI) test, however, a valuable procedure has become available for differentiating biologic false positive (BFP) reactions from those due to infection with T. pallidum. Because of the high percentage of BFP reactions observed among pregnant women with positive STS, a study was made to determine if pregnancy *per se* is a factor responsible for non-specific reactions. TPI tests were completed in sera from 400 pregnant and 400 non-pregnant women with positive STS. Two hundred eighty-four or 71 per cent of the pregnant women as compared to only 244 or 61 per cent of the non-pregnant women gave negative TPI reactions. Thus the number of

negative results in the pregnant group exceeds those in the non-pregnant group by 10 per cent, a statistically significant difference. In order to rule out possible primary syphilis at the time of the initial negative TPI result, second samples were submitted approximately three months later. Results varied in only one case in which an initially doubtful TPI test subsequently became positive.

Standard serologic tests for syphilis were performed on the same serum sample in 169 of the pregnant women who gave negative TPI results. Ninety-seven still gave positive reactions in one or more of the tests, and sixty-six had reverted to negative results. Anticomplementary reactions were obtained only in six specimens. The standard serologic tests for syphilis on serum from 295 pregnant women in whom the TPI gave negative results indicated a low degree of specificity for the serologic tests for syphilis.

DIAGNOSIS OF POLIOMYELITIS BY TISSUE CULTURE. *William M. M. Kirby* and Charles A. Evans.* Dept. Medicine and Microbiology, Univ. Washington School of Medicine, Seattle, Wash.

Utilizing trypsinized monkey kidney cells, stool specimens from more than sixty patients admitted to King County Hospital since July 1954, have been tested for the presence of poliomyelitis virus. Virus has been recovered from more than 80 per cent of the paralytic cases and from approximately 30 per cent of the non-paralytic cases. Of special interest are (1) the occurrence of an unusually large number of type III (Leon) infections and (2) the recovery from at least six patients of viruses which are not neutralized by any of the three types of poliomyelitis antisera.

IN VITRO SUSCEPTIBILITY OF PROTEUS TO ANTIBIOTIC AGENTS. *William L. Hewitt,* Charles B. Stone and Sidney Finegold.* Los, Angeles, Calif.

The pattern of antibiotic sensitivity of approximately 100 strains of proteus isolated from clinical sources has been studied employing a serial dilution technic. Accepting the fact that the sensitivity of proteus to any agent is not of a high order, two large groups have been delineated, (1) a penicillin-sensitive, broad spectrum antibiotic-resistant group (6 per cent of the strains in this group were sensitive to chloramphenicol but resistant to tetracycline) and (2) a penicillin-resistant, broad spectrum antibiotic-sensitive group. The relation of species classification to antibiotic sensitivity classification will be discussed. On a weight basis chloramphenicol was more effective than tetracycline. Fifty three per cent of the strains were sensitive to 12.5 μg. per ml. or less of the former agent and 19 per cent of the latter. Tetracycline was as effective or more so than oxytetracycline in all instances. Only 4 per cent of the strains were sensitive to neomycin or to streptomycin.

Because of the relative resistance of these bacteria to single agents, *in vitro* studies with combinations of antibiotics were undertaken. If one-fourth or less of the effective concentration of each single antibiotic would prevent growth when combined, a significant additive effect was assumed to occur. Among the penicillin-sensitive strains significant additive effects were observed with combinations of penicillin-neomycin (90 per cent of strains) and with chloramphenicol-neomycin (77 per cent) in spite of the relative resistance to neomycin alone. In many instances the increased antibacterial effect was quite striking. Among the penicillin resistant strains significant effects were observed with tetracycline-neomycin (83 per cent) and with chloramphenicol-neomycin (60 per cent).

PROTEOLYTIC ENZYME INHIBITION. *Frederic C. Moll.** Univ. Washington School Medicine, Seattle, Wash.

The proteolytic enzyme extracted from white blood cells is inhibited by serum. The variations in this serum inhibitor have been studied in Macacus rhesus monkeys. During control periods the serum inhibitor level is constant and of low magnitude. Upon stressing the animal by a sterile abscess, the inhibitor level promptly rises during the period of inflammation. A similar rise occurs following the administration of hydrocortisone followed by a slow fall to within normal limits. In adrenal insufficiency produced by sudden cessation of cortisone therapy, the sterile abscess is not followed by rapid rise of the serum inhibitor as under normal circumstances.

These observations suggest that the level of white cell proteolytic enzyme inhibitor level in serum is controlled in part through adrenal hormones and that it responds quickly to stress in the normal animal. This inhibitor response may explain in part the anti-inflammatory action of cortisone.

EFFECT OF ANTIBIOTICS ON INTESTINAL ABSORPTION OF VITAMIN B_{12} IN DISORDERS OF THE GASTROINTESTINAL TRACT. *James A. Halsted,**

Marian E. Swendseid, Marvin Gasster and Peter M. Lewis. Los Angeles, Calif.

By subtracting the fecal radioactivity, determined with a scintillation counter, from the amount present in a test dose of cobalt^{60}-labeled vitamin B_{12} administered orally, intestinal absorption of this vitamin can be determined with reasonable quantitative accuracy. The effect of various agents on absorption, such as intrinsic factor preparations or antibiotics, can be measured.

Using a test dose of 0.5 μg. of Co^{60}-B_{12} the average amount absorbed in eleven normal individuals was 68 per cent. In ten patients with various diseases of the small intestine 24 per cent was absorbed. In eight patients with pernicious anemia and in twelve patients who had had total gastrectomies there was essentially no absorption. Concomitant administration of intrinsic factor with the test dose had no effect in normal patients nor patients with small intestine disease, but in the pernicious anemia and total gastrectomy subjects absorption became normal in each instance.

The effect of aureomycin® or achromycin® on the absorption of vitamin B_{12} was determined in six patients with pernicious anemia, in four patients who had had total gastrectomies and in ten patients with small intestine disease. Two tests were performed in each patient during the administration of two grams of the antibiotic daily.

There was no effect on absorption in any of the patients with pernicious anemia, nor in any of the total gastrectomy group. Three of the patients with small intestine disease had megaloblastic anemia associated with intestinal anastomoses and stasis of intestinal contents. In these patients a very significant increase in absorption of Co^{60}-B_{12} occurred. Of the other seven patients two had non-tropical sprue, four had regional enteritis and one had steatorrhea due to pancreatic insufficiency. Although absorption of B_{12} was impaired in each of these seven, antibiotic administration did not result in increased absorption.

The relationship of antibiotics to vitamin B_{12} utilization and to hematopoiesis will be discussed in the light of these findings.

SERUM TRANSAMINASE LEVELS IN MYOCARDIAL INFARCTION. *Clarence M. Agress,* Albert A. Kattus and John S. La Due.* Cardiovascular and Cardiology Lab., Gen. Medical Research Service, V. A. Center; the Dept. of Medicine, Univ. California Medical Center, Los Angeles, Calif., and the Sloan Kettering Inst. Dept. Medicine, Memorial Center, New York, N. Y.

Clinical studies by one of the authors (La Due) have shown that the serum level of transaminase rises significantly following acute myocardial infarction. This work indicated that a study of this kind in dogs, where varied amounts of infarction could be produced and more accurately followed, would prove valuable. The coronary arteries of thirteen closed-chest dogs were embolized with plastic microspheres. Twelve-lead electrocardiograms were used to follow the course of the infections and color photographs were taken of the hearts at autopsy. Serum samples were taken every four hours for the first twenty-four to thirty-six hours and some dogs were followed for seventy-two hours.

The results showed that in all cases the serum transaminase level rose sharply after myocardial infarction, reaching a peak between twelve to eighteen hours after embolization. There was a relatively linear correlation between the peak serum transaminase level and the amount of myocardial infarction as estimated at autopsy.

A clinical study is being carried out at the Veterans Administration Center, Los Angeles; thus far 197 determinations have been made in sixty-four patients. Eleven were controls in which the highest level was twenty-four transaminase units. A miscellaneous group, including congestive heart failure, cerebrovascular accidents, old infarcts in five patients and pericarditis in four patients were in the normal range. Peak values of thirteen patients with acute myocardial infarction ranged from 40 to 580 transaminase units. In coronary insufficiency patients, as differentiated from myocardial infarction patients, serum transaminase levels were useful in fourteen of seventeen cases.

INFLUENCE OF BLOOD TRANSFUSIONS UPON THE CIRCULATING PLATELETS IN ARTERIAL AND VENOUS BLOOD OF MAN. *H. R. Bierman,* Keith H. Kelly, and Fauno L. Cordes.* Hosp. Tumors and Allied Diseases, Div. Research, City of Hope Medical Center, Duarte, Calif.

It has been shown by Harrington et al. that the blood or plasma of patients with idiopathic thrombocytopenic purpura (ITP) infused into volunteers will cause a thrombocytopenia in the venous blood. Prompt fall in the venous platelet level within fifteen to thirty minutes after the

infusion of the (ITP) factor has been confirmed. In three of five patients, however, despite significant reduction of platelet count in venous blood, the arterial platelet number was maintained at the original level for eight hours or longer, following which both the venous and arterial platelet level returned to similar levels. Infusion of whole blood from normal donors was followed by a reduction in the number of platelets, more marked on the venous side than on the arterial side. This reduction, however, was transient and returned to normal levels within three to four hours. The reduction in number of platelets was of the order of 50,000 to 100,000 platelets per cu. mm. In the average adult there are approximately 140 to 300 billion platelets that are readily available in the circulation. The significant changes in the platelet number encountered, therefore, reflect the disappearance of massive numbers of platelets from the circulation. The site of disappearance is probably related to the peripheral capillaries and to certain viscera.

DIURNAL VARIATION OF ADRENAL CORTICAL ACTIVITY IN MAN. *V. Di Raimondo.* Metabolic Unit for Research in Arthritis and Allied Diseases and Dept. Medicine, Univ. California School Medicine, San Francisco, Calif.

Diurnal variation in adrenal activity and response has been studied in two normal males and compared with that of two patients with abnormal adrenal states; one had a single hyperplastic adrenal gland and another had been on prolonged ACTH therapy. In the normal subjects steroid determinations of 17-hydroxycorticoids (17-OHC) and 17-ketosteroids (17-KS) were made in hourly urine specimens and blood 17-OHC levels were followed at four-hourly intervals.

From the data obtained the following conclusions may be drawn: (1) The diurnal variation in total urinary 17-OHC parallels that in the blood without any appreciable lag. This appears to exclude a renal phenomenon as the explanation for the observed diurnal variation in adrenal cortical activity. (2) Variations in liver function which parallel those in adrenal function are presumably not responsible for the diurnal changes in steroidogenesis observed, a conclusion suggested by the rise in the percentage of conjugated 17-OHC during the night period. (3) A diurnal variation in the adrenal response to exogenous ACTH has been demonstrated and shown to be due to an accelerated rate of steroidogenesis when the subject was stimulated with identical amounts of ACTH in the daytime and the nighttime. This suggests that the spontaneous diurnal variation in adrenal activity is not related to changes in the endogenous secretion of conventional ACTH.

The diurnal variation was found to be present in the abnormal adrenal states studied. It appears to be due to a change in intrinsic adrenal cortical responsiveness or capacity.

Fe^{59} KINETIC TRACER DATA SIMULATION WITH THE ELECTRONIC ANALOG COMPUTER. *Oliver J. Judd and Rex L. Huff.** Seattle V. A. Hosp. and Univ. Washington School of Medicine, Seattle, Wash.

An electronic analog computer (REAC-C302) was applied to simulate the kinetics of inorganic iron in man. Plasma iron tracer data from normal subjects and polycythemia vera and aplastic anemia patients were duplicated by the computer. The model used consisted of the inorganic iron of plasma, reticuloendothelial system, storage, red blood cell precurser and red blood cells connected by suitable rate constants representing exchange between these compartments. Results indicate smaller pool sizes than expected. More comprehensive data are needed. For normal subjects rate constants and rates of red cell turnover as determined are appropriate.

The application of this modern tool to facilitate computation in determining suitability of models and pool exchange rates to explain kinetic tracer data is useful and may have many uses.

USE OF RADIOACTIVE $CHROMIUM^{51}$ AS THE ERYTHROCYTE TAGGING AGENT IN DETERMINING BLOOD VOLUME AND IN VIVO ERYTHROCYTE SURVIVAL. *Merle M. Kurtz and Harold Tivey.* Div. Experimental Medicine, Univ. Oregon Medical School, Portland, Ore.

Using radioactive sodium chromate, erythrocyte survival time and total blood volumes were determined in ten normal females and eleven normal males. The plasma volume and erythrocyte mass were calculated from venous hematocrit readings. A sample of the subject's blood was incubated with 100 μg. of $chromium^{51}$, and a washed suspension of the tagged cells was injected intravenously. Using a whole blood sample, the circulating blood volume was calculated by the isotope dilution principle. The

mean points for the volume determinations with the 95 per cent range are as follows:

	Males	Females
Blood Vol. (ml./kg.)	60 (41.8–78.2)	56.2 (44.1–68.3)
Plasma Vol. (ml./kg.)	34.1 (23.6–44.6)	34.3 (25.5–37.1)
R.B.C. Mass (ml./kg.)	25.9 (18.1–33.7)	21.9 (16.6–27.2)

Red cell survival curves, when plotted on semi-log paper, appear to follow a straight line drop to about seventy days and then a more rapid curvilinear drop occurs toward zero activity. Mathematical analysis of such curves shows promise of obtaining information which will estimate the total survival time, the elution factor and perhaps a factor of random erythrocyte destruction.

Blood volume determinations by this method have the advantage of measuring only intravascular space and offer a practical method for repeated blood volume studies over a twenty-four-hour period in the absence of blood loss. Because of the low radiation dosage to the patient blood volumes can be repeated at intervals by repeating the procedure.

INTRACAVITARY ADMINISTRATION OF TEM IN PATIENTS WITH EFFUSIONS DUE TO MALIGNANT PLEURAL IMPLANTS. *J. F. Mangum, B. E. Hall* and F. M. Willett.*

Effusions due to malignant pleural implants respond poorly to x-ray therapy and parenteral and oral chemotherapy. Intrapleural nitrogen mustard and more recently radioactive colloidal gold and chromic phosphate have been used to diminish the formation of fluid. During the past two years we have investigated the effects of the intracavitary administration of TEM in patients with pleural effusions due to lymphosarcoma, Hodgkin's disease and mesothelioma. The effect of triethylene melamine on the normal pleura of guinea pigs was studied also. This report includes three cases of lymphosarcoma, four cases of Hodgkin's disease and one case of mesothelioma with the following results: one case of lymphosarcoma with complete arrest of fluid formation for twenty-four months; one case of mesothelioma with arrest of fluid formation for eight months; one case of lymphosarcoma with definite decrease in fluid formation but no prolongation of life; three cases of Hodgkin's disease with some palliation of fluid formation but no prolongation of life, and one case each of lymphosarcoma and Hodgkin's disease with no alteration in fluid formation.

Intrapleural TEM caused no adhesions or pleural thickening in normal guinea pigs.

RACIAL AND INDIVIDUAL DIFFERENCES IN THE PERIPHERAL VASCULAR RESPONSE TO A COLD STIMULUS. *John P. Meehan.* Arctic Aeromedical Lab., Ladd Air Force Base, Alaska and Dept. Physiology, School Medicine, Univ. Southern California, Los Angeles, Calif.

Studies conducted in Alaska on groups of both white and Negro military personnel as well as on groups of Alaskan natives indicate that during immersion of the hand in ice water the Alaskan natives maintained a greater circulation to the fingers than did those subjects in the other two groups. A group of individuals who had experienced injury from the cold were similarly studied and they maintained the poorest circulation to the hand and fingers of all subjects studied. Skin temperatures as measured with thermocouples were used to indicate the magnitude of the cutaneous circulation during the exposure of the hand to ice water. Plasma volume estimations by the T-1824 method were made in all subjects studied. In general, subjects maintaining the highest skin temperatures of the hand exposed to cold had the greatest plasma volume.

A PRECIPITIN TEST FOR SERUM LIPOPROTEINS IN HUMAN CORONARY ATHEROSCLEROSIS. *Lester M. Morrison,* Monica Stevens and Hyman C. Bergman.* Los Angeles County Hosp. and College of Medical Evangelists, Los Angeles, Calif.

Recent studies by Kunkel, Gitlin, Baker and Grant and Berger have demonstrated that a simple serologic precipitin test can demonstrate the various serum lipoprotein classes in human subjects as described by Gofman. This precipitin test is accomplished by immunizing rabbits with lipoprotein fractions at Svedborg flotation rates of S_F 10–100 and S_F 10–400. The immune rabbit antiserum was then employed to detect lipoprotein concentrations in a series of forty-four human subjects (a) with proven coronary thrombosis and myocardial infarction as exemplary of atherosclerosis of the coronary arteries, (b) with seventeen so-called "normal" controls and (c) with twenty-four patients with miscellaneous diseases not affecting the cardiovascular system or disorders in lipid metabolism.

It was found that statistically significant dif-

ferences in precipitin test reactions to serum lipoproteins were found in human coronary atherosclerosis and control subjects.

CYTOLOGY OF CARCINOMA DEVELOPING IN THE GASTRIC ATROPHY OF PERNICIOUS ANEMIA. *Cyrus E. Rubin and Barbara W. Massey.* Univ. Washington School Medicine, Seattle, Wash.

Characteristic large columnar cells are shed from the gastric mucosa of patients with pernicious anemia. These cells are twice that of normal size. They are found regardless of whether or not the patient has been treated and probably reflect the severe and irreversible gastric atrophy of this disease. Most of these large cells are bland in appearance but some show morphologic indications of active growth bordering on malignancy. This is of interest since it is well established that gastric polyp and carcinoma are more frequent in this illness.

Exfoliative cytology established the diagnosis of gastric carcinoma in three elderly men who had been treated for pernicious anemia of more than twenty years' duration. Histologic confirmation was obtained by various methods, namely, gastric suction biopsy, laparotomy and necropsy. The spectrum of gastric cellular morphology was remarkably wide and varied from large bland cells through large cells of increasing activity to frank carcinoma. The morphologic evidence strongly suggested that the large columnar cell was the cancer progenitor.

This small series merits no conclusions but it poses questions demanding further study: Where do the large cells come from? Do they originate in the islands of intestinal metaplasia which dot the atrophic gastric mucosa? Will this large cell be found in certain other gastric carcinomas? Preliminary attempts to answer these questions by light, phase and electron microscopy will be described.

METABOLISM AND EXCRETION OF INTRAVENOUSLY INFUSED SORBITOL. *Victor P. Seeberg and Ernest B. McQuarrie.* Research Div., Cutter Labs., Berkeley, Calif.

We have previously observed, as have others, that intravenous administration of the hexahydric alcohol, sorbitol, causes a sharp rise in reducing substances in the blood, indicating rapid conversion to hexose. The present work was directed toward identifying the primary conversion product or products. The over-all objective is the continued exploration of the possible usefulness of sorbitol as a carbohydrate for parenteral alimentation with amino acids. Having no reducing groups, it does not participate in the Browning reaction during autoclaving and storage with the latter.

Unanesthetized rabbits were infused intravenously with sorbitol solution at constant rates and total doses approximating clinical rates and doses. Blood and urine were studied. Infused sorbitol was found to undergo considerable immediate conversion to fructose. There was also some evidence of delayed conversion to glucose. Utilization varied from 80 to 93 per cent and was dependent upon the rate of administration. Excretion consisted mainly of unchanged sorbitol with traces of reducing substances.

CHANGES IN STANDARD LEAD I OF THE ELECTROCARDIOGRAM AFTER INGESTION OF GLUCOSE. *Reider Wennesland, Rosemarie Lenel and Alfred A. Bolomey.** Cardiovascular Clinic of Kaiser Foundation Hospital, Oakland, Calif.

During a "multiphasic health check" program a number of abnormal lead I electrocardiograms were observed. On followup study many of these had reverted to within normal limits. The routine of this program involved taking the electrocardiogram one-half hour after ingestion of 100 gm. of glucose. The present study was undertaken to determine whether these "false positive" electrocardiograms might not be associated with glucose ingestion. A lead I electrocardiogram was obtained from 495 men and 499 women before and after ingestion of 100 gm. of glucose. The most common changes observed following ingestion of glucose were prolongation of the Q-T interval, lowering of the T wave, rounding of the junction and depression of the S-T segment.

In the male group the mean Q-T ratio changed from 0.964 to 0.993 with a standard error of the difference of ± 0.00215. In the female group the Q-T ratio changed from 0.991 to 1.017 with a standard error of the difference of ± 0.002. Thus in both sexes the difference of the means, 0.029 and 0.026 respectively, was thirteen times the standard error of the differences. Lowering of the T-waves was found in 27.1 per cent of the male and 33.4 per cent of the female subjects. Other changes were found in 9.9 per cent of the male and 3.8 per cent of the female subjects.

In twenty-eight males and thirty-one females

the changes led to a change of interpretation from normal to abnormal results.

INSULIN ACTION ON GLUCOSAMINE. *Arne N. Wick* and Douglas R. Drury.** Scripps Metabolic Clinic, La Jolla, and Dept. Physiology, Univ. Southern California, Los Angeles, Calif.

Insulin increases the transfer rate of glucose, galactose and mannose from the extracellular fluid into the cells of the extrahepatic tissues. Other compounds related to glucose, such as sorbitol, fructose, gluconic acid and 3 methylglucose, do not enter the muscle cells to any measurable extent even in the presence of insulin action.

In a continuation of these studies we have recently examined glucosamine, an amine derivative of glucose, which is found combined in many body constituents. This amine responds like glucose to insulin administration. Upon entering into the process accelerated by insulin, glucosamine inhibits the transfer of glucose.

EFFECTS OF ABNORMALITIES OF THE NERVOUS SYSTEM ON PERIPHERAL CIRCULATION. *Travis Winsor* and Nicholas Khoury.*

The purpose of this report is to show the effects of organic and functional abnormalities of the nervous system on peripheral circulation and to show the diagnostic value of recognizing these circulatory aberrations. The studies were carried out on 125 normal individuals and on forty-five patients with various diseases. The pneumoplethysmograph was employed to record consecutively the vasomotor reactions from one digit of each extremity. A recording thermocouple was employed to record skin temperatures of the digits and the Lang sphygmotonograph was used to record blood pressures. Certain reflexes studied were (1) the induced and spontaneous digital vasomotor reflexes recorded with the patient supine and (2) postural cutaneous vascular reflexes and systemic blood pressure reflexes recorded with the patient supine and standing. The induced vasomotor reflexes each with a different afferent nerve pathway included the inspiratory (vagus nerve), the sound-startle (acoustic nerve), the light (optic nerve) and the pain reflex (sensory nerve). The efferent pathways for these reflexes are from the medulla over the sympathetic system to the blood vessels. It was found that: (1) a lesion in one of the four receptor organs (lung, eye, ear and skin) or in its afferent pathway prevented the normal constriction of the digits of the four extremities following stimulation of the diseased organ or nerve, while stimulation of the three normal organs produced normal vasoconstriction in the digits of each extremity. (2) Lesions of the brain (medulla) resulted in an absence of vasoconstriction in the digits of all extremities when the four receptor organs were stimulated consecutively. This was seen with syringomyelia, and after reserpine and the D-H alkaloids of ergot. (3) Lesions of the upper thoracic and lumbar sympathetic ganglia (for example, quadrilateral sympathectomy) or drugs acting on the sympathetic ganglia (for example, tetraethylammonium chloride) resulted in absence of the reflexes in all digits when any one receptor organ was stimulated. (4) Localized lesions of the sympathetic nervous system in the region of the cervical or lumbar sympathetic ganglia produced localized inhibition of digital reflexes of the arm or leg, respectively, when any receptor organ was stimulated. This occurred in patients with severe diabetic neuritis and resulted in localized absence of reflexes in a digit after stimulation of any receptor organ. A posterior tibial nerve block with novocain produced similar results. In addition to the reflex changes previously described disease of the brain or peripheral sympathetic system resulted in disappearance of the spontaneous vasomotor activity which was often coincident with an increase in amplitude of the pulse waves. With disease of the brain a rise in systolic and diastolic blood pressure was not common after blowing against a 20 mm. column of mercury for 20 seconds. With localized lesions in the sympathetic system the normal fall in skin temperature of the digits (due to vasoconstriction) failed to occur when the patient stood.

Case Reports

Agammaglobulinemia in the Adult*

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AGAMMAGLOBULINEMIA is a unique syndrome characterized by a specific defect of a plasma protein component defined by the electrophysical pattern of the serum proteins. In recent years this defect has been discovered in several children who were subject to recurrent multiple infections.

One of the first pediatric cases of agammaglobulinemia reported was by Bruton in 1952.[1] The patient was an eight year old boy who in four years had had at least nineteen episodes of clinical sepsis in which some type of pneumococcus was recovered from his blood on ten occasions. He also had had epidemic parotitis three times. Subsequently Keidan et al.[2] diagnosed agammaglobulinemia in a fatal case of generalized vaccina in a seven week old girl. Recently, Janeway, Apt and Gitlin[3] have collected data on eight male children with agammaglobulinemia, all of whom were subject to recurrent multiple infections. These children were unable to produce circulating antibodies The absence of serum isohemoagglutinins in patients of blood groups O, A or B served as a simple screening test for this condition. The monthly injection of 0.1 gm. per kg. of gamma globulin from the serum of a normal person gave these patients considerable protection against recurrent infections.

Other examples of "idiopathic" defects in the serum proteins, with the principal manifestation of edema due to a lowered serum albumin level, have been reported.[4–15] Lowered serum globulins were found in some of these. In the few cases studied by electrophoresis the gamma globulin has been within normal limits or considerably diminished[5–7] but never absent, and repeated infections were not apparent. In one well studied case[7] increased destruction rather than diminished production of serum proteins was noted. A decrease in gamma globulin has also been reported in patients with nephrosis[16] or malnutrition[17] with edema.

For three years we have had in our care a fifty-two year old white man with agammaglobulinemia who has experienced repeated infections of the respiratory tract and several episodes of severe diarrhea. Recently we diagnosed agammaglobulinemia in a forty year old white man who had had no difficulty with respiratory infections but had had repeated intestinal difficulties.

The authors know of eleven additional cases of agammaglobulinemia in the adult, of which four are women. Allen[18] has treated a middle-aged woman with agammaglobulinemia for eight years with several hundred plasma transfusions. During this time the patient underwent successful pregnancies. Sturgis[19] recently has observed a thirty-six year old man with this serum defect who suffered from repeated infections. Zinneman[20] has under observation three adults who have no detectable gamma globulin. One of these is a woman with granulomatous disease; an enlarged spleen has been removed. Young and Wolfson[21] have described four adult men with agammaglobulinemia and extreme susceptibility to infection, three of whom had no lymph node germ centers. Arends et al.[22] found no gamma globulin in a fifty-three year old woman who had a mild form of malignant lymphoma with repeated infections of the skin and respiratory tract. The bone marrow contained foci of abnormal lymphocytes; no plasma cells were present. Sanford et al.[23] have recently described a thirty year old house-

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wife with this abnormality which was manifested by repeated infections and a sprue-like syndrome.

CASE REPORTS

CASE I. Patient J. N., a fifty-two year old executive, had been observed since March, 1951. Past history included a period of dermatitis on the back of his hands in 1927, followed shortly thereafter by a series of furuncles occurring periodically for five months. In 1931 he had bronchial asthma resulting from sensitivity to aspirin. In 1945 cholecystectomy was performed after an attack of acute cholecystitis. A short time later the common duct was explored because of recurrence of jaundice but no stone was found.

The first of the present infections began in January, 1950, with an eye infection for which he received three injections of penicillin. This was followed by eruptions on his face, hands, scrotum and legs, lasting two weeks. A different type of eruption then occurred consisting of small, pink, raised nodules, ½ to 2 cm. in diameter. The centers of many of these became necrotic, sloughed off, and ultimately healed, leaving small pigmented scars. Small numbers of these lesions continued to occur over the face, trunk and extremities. A course of staphylococcus toxoid and vaccine for two months proved ineffectual in controlling these lesions, but with penicillin therapy they healed completely. After administration of prophylactic penicillin, given twice weekly since July, 1950, there were only a few brief recurrences. In January, 1951, he had a severe attack of herpes zoster on the right lower flank which became gangrenous but healed with scarring. For three months prior to March, 1951, he had a cough producing small amounts of mucoid to mucopurulent sputum. He had lost 15 pounds.

When first seen in March, 1951, his temperature was 98.4°F., pulse 80, blood pressure 130/80. Other than a widespread rash as previously described, the only positive physical findings consisted of an atrophic nasal mucosa, a few rales at the posterior base of the left portion of the lung and a firm enlarged spleen descending three cm. below the left costal margin. A hemogram showed a red blood count of 5.36 million per mm.^{3}, hemoglobin 15.0 gm. per cent, a white blood count of 7,950 per mm.^{3} with a differential of 6 eosinophiles, 9 stab forms, 39 segmented forms, 42 lymphocytes and 4 monocytes. The reticulocytes were 2.2 per cent and the platelet count was 308,000 per mm^3. Urinalysis, Kahn test, fasting blood sugar test, electrocardiogram, cephalin cholesterol flocculation test and thymol turbidity test all gave normal results. Three of four stools showed occult blood. A complete examination of the gastrointestinal tract revealed it to be normal except for postoperative changes in the biliary tract and displacement of intestinal loops by the enlarged spleen. Chest roentgenogram showed honeycombing and diffuse interstitial infiltration of the left upper, left lower and right middle lobes, suggestive of bronchiectasis; fibrocalcific tuberculosis of the left upper lobe, and slight ventricular enlargement on the left side. Serum proteins contained 5.7 gm. per cent albumin and 0.2 gm. per cent globulin revealed by the methanal precipitation technic[24] and 4.8 gm. per cent albumin and 0.9 gm. per cent globulin by the salt precipitation technic. Electrophoretic analysis (Table I) revealed no gamma globulin. A test for cryoglobulins gave negative results. Bone marrow biopsy (Table II) showed normal cell marrow with slight erythroid hyperplasia. A quantitative plasma cell count showed only one plasma cell per 5,000 nucleated cells.[25] The tuberculin skin test gave negative results.

TABLE I
ELECTROPHORETIC STUDIES ON SERUM

Date	Total Protein (gm. %)	Albumin (%)	Globulins (%)				A/G Ratio
			Alpha-1	Alpha-2	Beta	Gamma	
4/4/51*	....	55.3	9.7	18.0	17.0	0	1.23
4/4/52	5.01	55.3	9.8	17.5	17.3	0	1.23
11/12/52	....	54	13	19	14	0	1.17

* Made by Dr. John Taylor; the other determinations were done by Dr. S. Howard Armstrong, Jr.

The nodular rash recurred in July, 1951. Skin biopsy at that time showed slight acanthosis of the epidermis. The dermis was edematous, and in the papillary portions the blood vessels and lymph spaces were dilated. A moderate amount of perivascular infiltration of lymphocytes and histiocytes, with some polymorphonuclear leukocytes were noted. No definite diagnosis could be made.

In May, 1951, the patient had a series of acute infections of the lung and respiratory

tract (Table III). Between attacks a chronic, productive cough persisted. It is to be noted that, in spite of severe infections with high fever and at times lobar consolidation, the white blood count did not rise but usually a marked shift to the left in the differential count was noted. The

TABLE II
BONE MARROW DIFFERENTIALS

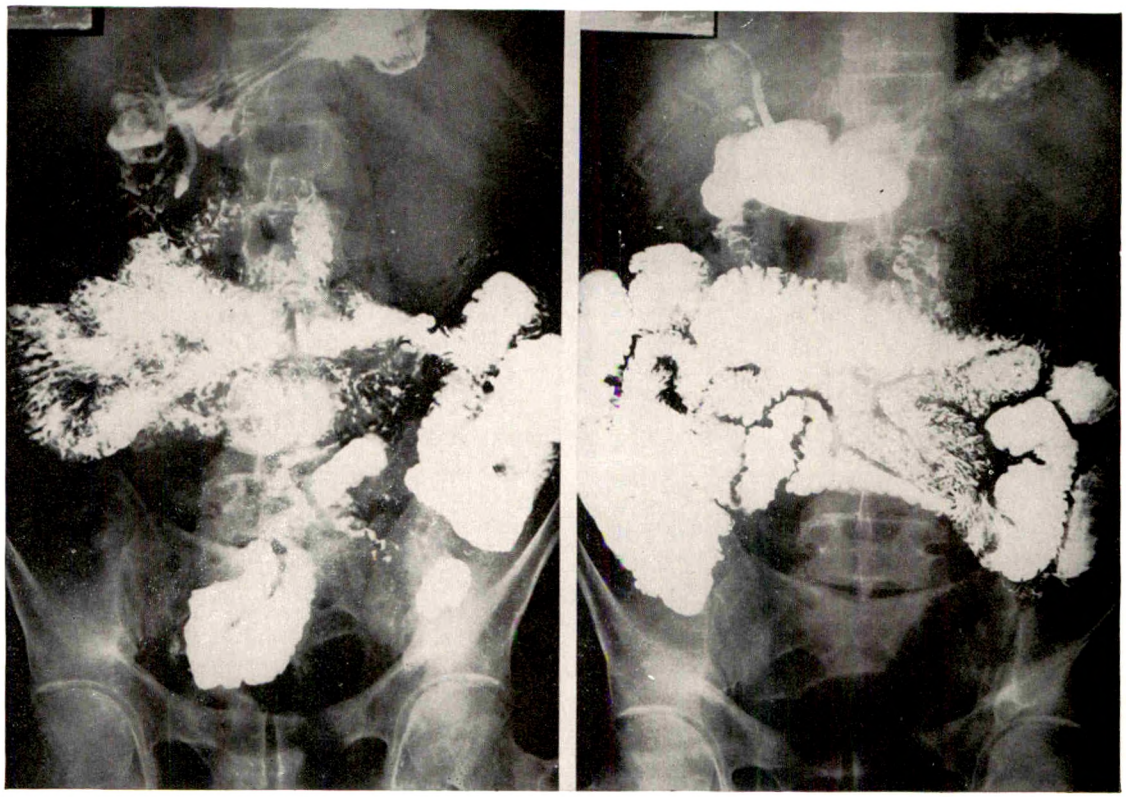
Cell Type	Patient J. N.		Patient D. S.
	March 1951	October 1953	April 1947
Megakaryocytes	1.0	1.5	1.0
Segmented forms	17.5	21	17
Stab forms	24	25.5	25.5
Metamyelocytes	10	13.5	11
Myelocyte "c"	28.5	22.5	29.5
Promyelocyte	0.5	0	0
Basophilic leukocyte	0.5	0	0
Eosinophilic leukocyte	5	0	4
Clasmatic phagocyte	0.5	0	0
Lymphocyte	9	13	10
Plasma cells	0.5	0	0
Reticulum cells	0.5	3	2
Total %	100.0	100.0	100.0
Nucleated RBC/100 WBC			
Normoblasts	54.5	81.5	42
Late erythrocytes	7.5	4	8
Early erythrocytes	1.0	4	3
Total	63.0	89.5	53

infections always responded promptly to chemotherapy and usually to penicillin alone. Physical examination revealed essentially no changes. Blood counts are recorded in Table III.

On December 9, 1951, twenty-one days after completing a course of two grams of oxytetracycline a day for six days, the patient had a sudden onset of sharp abdominal pains, fever and diarrhea with seven to ten loose watery stools daily without mucus or blood. No response resulted from administration of the usual antidiarrheal medications, and he was hospitalized on December 25, 1951. Physical examination revealed no changes except for evident dehydration. Sigmoidoscopy showed a pink, friable mucosa that bled upon slight trauma. One large ulcer was present in the anal canal. Stools contained no parasites or bacterial pathogens. Roentgenograms of the gastrointestinal tract revealed a hiatus hernia, numerous narrowed segments of small intestine with distorted mucosal pattern, and a transient jejunal intussusception. (Fig. 1A.) A barium enema gave normal results. A tentative diagnosis of acute diffuse, regional enteritis was made. While on prophylactic chemotherapy, the patient was treated for one week with ACTH, 100 mg. administered intramuscularly daily.[26] The diarrhea continued but the spleen decreased in size. Because of progressive malnutrition and debility, he was given high protein, high fat tube feeding which increased the diarrhea until there were ten to twenty bulky stools daily. He developed a hypokaliemic, hypochloremic alkalosis. On a regimen of parenteral feedings exclusively, the diarrhea subsided and the electrolyte disturbances were corrected with retention of large amounts of potassium.

During the next few weeks he was placed on a low residue diet, treated with banthine® and B_{12} injections, and in February the diarrhea gradually decreased to two or three movements daily. Studies of intestinal absorption showed a flat oral glucose tolerance curve (fasting 60 mg. per cent, one hour 102 mg. per cent; two hours 104 mg. per cent; three hours 116 mg. per cent). Fasting plasma carotene level was 34 gamma per cent (normal range 70 to 200 gamma per cent). Vitamin A tolerance showed a rise from 65 gamma per cent fasting to only 90 gamma per cent in four hours after oral administration of 300,000 units of vitamin A palmitate in oil.[27] Blood prothrombin time, calcium and amylase levels were within normal limits. The patients hemoglobin had fallen to 10 gm. per cent, and studies showed the anemia to be normochromic. Stool analysis (Table IV) showed upper normal or slightly increased fat excretion, but greatly increased nitrogen excretion.[28] Repeat roentgenographic studies of the small intestine on January 30, 1952, gave normal results. (Fig. 1B.)

Further studies (by electrophoresis (Table I) and by an immunologic technic) of the serum proteins still showed no gamma globulin. The patient's blood type was group O, Rh positive, but he had no A or B isoagglutinins in his blood. After intravenous injections of AB Witebsky substance,[29] no A or B isoagglutinins were detected. Indirect Coombs tests on the patient's serum during this period gave negative results,



1A 1B

FIG. 1. A, a roentgenogram of the small intestine taken on December 29, 1951, showed distorted mucosal patterns and irregular spacing of loops in the distal jejunum and ileum three quarters of an hour after barium ingestion. Later, films showed string signs in the jejunum. There is an intussusception near the distal jejunum which proved to be transient. Barium is seen within the common bile duct due to postoperative abnormalities. A hiatus hernia, a duodenal diverticulum and displacement of intestine due to the enlarged spleen were also present. B, a small intestinal film was taken at one and one-half hours on January 30, 1952. No abnormality of the jejunum or ileum could be demonstrated. The bladder is distended.

making unlikely the presence of incomplete antibodies. The patient's total serum complement was 77 to 50 per cent hemolytic units (simultaneous normal value was 57 to 50 per cent hemolytic units). Determination of plasma and urinary amino acids by paper chromatography revealed a normal spectrum but there were unique spots in the urine and stool which migrated in the same manner as ethanolamine conjugated with phosphoric acid.

Mild diarrhea of two to three semisolid stools a day persisted only a short time after the patient left the hospital in March, 1952. During the spring of 1952 and during the following winter he had had numerous episodes of pneumonia treated with antibiotics and divided doses of gamma globulin. (Table III.) One attack of pneumonia in November 1952 was complicated by a sterile pleural effusion which contained 7,600 red blood cells/mm.^{3}, 1,250 white blood cells/mm.^{3}, 70 per cent polymorphonuclears and a protein content of 3.1 gm. per cent.

In February, 1953, the patient again had a sudden onset of six to ten loose, watery stools a day with cramping, and the pustular skin rash on the face recurred. He lost 12 pounds. The usual antidiarrheal agents again were of no help. He was given penicillin, gamma globulin injections, banthine and cortisone, the latter in dosages starting with 200 mg. per day and gradually decreasing to 100 mg. per day. Within a week the skin rash cleared, the diarrhea subsided to two semiliquid stools daily and the patient began to regain weight.

During the next eight months he had several episodes of skin rash, sinus and pulmonary infections, as recorded in Table III, for which

TABLE III

SUMMARY OF CLINICAL COURSE (PATIENT J. N.)

Year	Month	Infection	Temp. °F	White Blood Count						N.	Treatment
				Total	Eo.	Juv.	Stab.	Seg.	Lym.		
1950	1	Eye infection	.....	.....	..	..	..	..	..	..	Penicillin
	3	Skin rash	.....	.....	..	..	..	..	..	..	Staphylococcus vaccine
	6	Cellulitis, hand	.....	.....	..	..	..	..	..	..	Penicillin
	9	.....	.....	.....	..	..	..	..	..	..	Prophylactic penicillin
1951	1	Herpes zoster	.....	....		..	..	..	..	..	.....
	2	Right flank	.....	7,200	..	..	3	66	30	1	.....
	3	.....	.....	7,950	6	..	9	39	42	4	.....
	5	Pneumonia L.L.L.	101.6	7,750	..	..	..	..	..	..	Penicillin, chloro-tetracycline
	7	Skin rash	98.2	5,450	5	..	3	41	51	..	Penicillin
	9	Upper respiratory infection	97.6	9,000	4	..	..	57	38	1	Penicillin, chlor-tetracycline
	10	Acute bronchitis and sinusitis	102.5	5,700	..	..	..	..	..	..	Oxytetracycline, 1 week, antral washing
	11	Acute sinusitis	102	6,750	..	..	16	47	36	1	Penicillin and oxy-tetracycline, 1 week
	12	R.U.L. pneumonia	103	7,800	..	..	54	14	19	14	Penicillin
1952	1	Diarrhea and rash, R.L.L. pneumonia	101	....	..	..	..	..	..	..	Banthine, ACTH, etc., penicillin
	2	L.L.L. pneumonia	102	....	..	..	..	..	..	..	Penicillin
	3	R.L.L. pneumonia	101	4,950	6	..	14	43	30	7	Penicillin, chlor-tetracycline
	5	L.L.L. pneumonia	101	6,800	2	..	25	33	32	8	April 16–May 7, 3.2 gm. gamma globulin 1 M., penicillin, chlortetracycline
	6	.....	.....	8,100	6	3	17	26	40	8	Feeling well
	7	.....	.....	4,200	..	..	16	42	40	2	.....
	11	L.L.L. pneumonia	103	9,200	..	2	51	25	21	1	Penicillin, Nov. 12–Jan. 13, 1953, 5.42 gm. gamma globulin, penicillin + thoracentesis
	12 and 1/53	L.L.L. pneumonia w. sterile pleural effusion	101+	4,950 5,300	.. 3	.. ..	.. 15	.. 41	.. 36	.. 5	
1953	2	Diarrhea and skin rash	.....	5,800	1	..	23	45	29	2	Penicillin, cortisone, banthine, etc., Feb. 10–15, 3.8 gm. gamma globulin

TABLE III (*Continued*)

Year	Month	Infection	Temp. °F	White Blood Count						N.	Treatment
				Total	Eo.	Juv.	Stab.	Seg.	Lym.		
1953	3	Acute bronchitis and sinusitis; Friedländer's bacillus in sputum; ankle edema; local swelling of dorsum of hands	101	4,100	1	..	19	35	399	3	Penicillin, sulfadiazine, 2.3 gm. gamma globulin, March 4–19
	4	Contact dermatitis of hands and back; pustular rash of back	.....	4,300	..	..	..	..	...	..	Local therapy
	5	Acute sinusitis	100	4,100	..	..	..	..	...	..	Penicillin, triple sulfa
	7	R.L.L. + L.L.L. pneumonia; alkaligenes fecalis, Staph. aureus and hemophilus	101.8	3,900	2	..	6	8	80	4	Penicillin, streptomycin, July 25–Aug. 10, 8.7 gm. gamma globulin
	8	Type A Friedländer's bacillus	100.6	3,900	1	1	24	15	53	3	Triple sulfa, oxytetracycline
	10	R.L.L. pneumonia, alpha + beta strep, Neisseria	101	6,950	1	..	23	39	31	4	Penicillin 1.3 gm., gamma globulin, triple sulfa, tetracycline
			.....	5,150	2	1	7	47	37	6	
	11	Spleen, larger and tender	.....	5,000	2	..	22	23	50	2	Splenic puncture,
	12		.....	1,550	2	1	10	16	70	1	X-ray to spleen, 450 roentgens
1954	1	Slight cough; postnasal drip; spleen smaller	.....	4,100	6	1	24	31	35	3	From Nov. 30–Dec. 8, 3.9 gm. gamma globulin
	6	Well; spleen larger	.....	4,000	5	..	19	39	34	3	.96 gm. gamma globulin per week, Jan.–June

he received antibiotics and gamma globulin injections. During the infections of July, 1953, a transient, profound granulocytopenia was observed. In March, 1953, a non-tender tenosynovitis appeared on the dorsum of both wrists which subsided in several months.

In April, 1953, he received 500 microcuries of S-35 labeled yeast, and the albumin and globulin turnover time was determined by Dr. S. H. Armstrong, Jr.[30] The results of the test were as follows: albumin turnover time, thirty-five days; mixture of alpha$_1$, alpha$_2$, beta globulin turnover time, eight days; gamma globulin turnover time, no radioactivity recovered in the gamma portion on paper. These turnover times are either in the upper normal range or are slightly elevated.[31]

In October, 1953, the patient's spleen was tender and became larger. It extended 11 cm. below the left costal margin, and the liver edge was felt 3 cm. below the right costal margin. At that time the red blood count was 5.17 million/mm.^{3} hemoglobin, 12.7 gm. and platelets, 435,000/mm.^{3} The white blood count, the differential count and results of bone marrow examination are recorded in Tables II and III.

Blood non-protein nitrogen, fasting sugar, and liver function tests gave normal results.

He was admitted to the hospital. A splenic puncture was performed. Microscopic sections revealed atrophy of malpighian follicles, hemosiderosis, rare megakaryocytes and myelocytes,

TABLE IV
STOOL FAT AND NITROGEN EXCRETION

Period	Fat			Nitrogen		
	Intake (gm./day)	Output (gm./day)	Excretion (%)	Intake (gm./day)	Output (gm./day)	Excretion (%)
2-2-52 to 2-6-52	90	10.5	11.5	10.6	3.1	28.8
2-16-52 to 2-20-52	120	8.7	7.4	....	...	....
Upper normal values[21]	102	6.7	6.8	18.8	2.5	13.3

with questionable stem cells in the pulp and a questionable increase in reticulum cells. Because of the size of the spleen and the possible role of secondary hypersplenism in the hematologic picture, it was decided to irradiate the spleen. After 450 roentgens in eleven days, the procedure was terminated because of a granulocytopenia which proved to be self-limited. The spleen shrank to 7 cm. below the left costal margin. From December to June, 1954, he received 960 mg. of gamma globulin intramuscularly weekly without recurrence of active infection. However, a postnasal drip and a slight productive cough persisted for several months. The spleen has gradually increased to its former size.

CASE II. Patient D. S., a forty year old oil field superintendent, was first seen in 1947 when he was thirty-three years old. He had an eight-week history of severe and frequent vomiting following ingestion of food. For about a year and a half he had frequent bloating, heartburn and heaviness in his epigastrium, with occasional vomiting and intolerance to fatty foods. There had been one period of bright red blood in the vomitus. He had lost 30 pounds.

Physical examination revealed the temperature and pulse to be within normal limits. The blood pressure was 92/52. He was poorly nourished. The only positive physical observation was abdominal tenderness just above the umbilicus. None of the organs was palpable, and muscle guard was absent.

Laboratory data revealed a hemoglobin of 13.0 gm. per cent. The white blood count was 5,300/mm.^{3}; the differential blood count showed 60 segmented forms, 34 lymphocytes, 4 monocytes and 2 eosinophils. The reticulocyte count was 3.7 per cent and the platelet count was 119,000/mm.^{3} Serum non-protein nitrogen was 13 gm. per cent, fasting blood sugar 74 mg. per cent, amylase 95 units per cent, albumin 3 gm. per cent and globulin 1.2 gm. per cent. Examination of the gastrointestinal tract showed narrowing in the second portion of the duodenum approximately 8 cm. from the duodenal bulb, at which point the duodenum was only ½ cm. in diameter. It was believed this was either a narrowing due to adhesions or to an annular pancreas.

At surgery an annular pancreas was found. A number of enlarged lymph nodes were present at the base of the mesentery. Microscopic sections of the lymph nodes showed nonspecific chronic inflammation. The patient, feeling well, was discharged. He was readmitted ten days later because of three days of loose watery stools and one day of upper abdominal colic and vomiting. Bone marrow examination (Table II) revealed one plasma cell per 5,000 nucleated cells.

On admission his temperature was 37.2°C.; blood pressure was 112/60. Slight left axillary lymph node enlargement was noted. One observer thought the liver was enlarged. Laboratory study revealed the urine to be within normal limits except for an occasional red cell and white cell. His hemoglobin was 10.6 gm. per cent. The white blood count was 4,000/mm.^{3} and the differential count revealed 62 segmented forms, 1 stab form, 32 lymphocytes, 3 eosinophils and 1 basophil. Serum non-protein nitrogen was 13 mg. per cent, icterus index 5 and amylase 66 units per cent. Cephalin flocculation test gave negative results; thymol turbidity test revealed 1.2 units, and a bromsulfalein test revealed no retention in 45 minutes. Prothrombin time was 47 per cent; clotting time was six minutes; red cell fragility showed beginning hemolysis in 0.43 per cent saline solution and was complete in 0.32 per cent saline solution. Two determinations of serum proteins showed albumin to be 2.8 and 3.3 gm. per cent, and globulin to be 1.2 and 1.3 gm.

per cent. Two stools gave negative results to the guaiac test, and one stool was described as showing some increase in fat. Studies of the upper gastrointestinal tract revealed a filling defect resulting from external pressure on the antrum and the distal part of the greater curvature of the stomach. The duodenal loop was described as being partially widened but there was no obstruction in the duodenum. The proximal small intestinal loops were atypical and slightly dilated, and there was some question as to partial small bowel obstruction. A barium enema gave normal results. During this hospital stay biopsy revealed chronic inflammation of a cervical lymph node. A later review of this and the previous abdominal lymph nodes revealed absence of the germinal follicles.

The patient gradually gained weight. Blood tests showed consistently normal hemoglobin, white counts were between 4,000 and 6,000, differential counts gave normal results and the urine was normal. Sedimentation rate was 4 mm. an hour. Splenomegaly was first detected in December, 1947.

The patient was admitted for the third time on March 11, 1954, because of three days of diffuse abdominal pain with severe waves of colic every five or ten minutes. He felt better in a sitting than in a lying position; some concomitant abdominal swelling was present. His bowels moved normally. On the day before admission he vomited a great deal. His temperature was 37°C., his pulse 80 and his blood pressure 120/70. His abdomen was distended and tender throughout the right half. It was thought the patient had upper bowel obstruction and this was confirmed by a flat film of the abdomen.

Laboratory analysis revealed a hemoglobin of 14.6 gm. per cent; the white blood count was 6,600 with 69 segmented forms, 8 stab forms, 21 lymphocytes and 2 monocytes. The urine was within normal limits. A trace of albumin and 2 to 4 white cells per high power field were present. Serum non-protein nitrogen was 30 mg. per cent. Fasting blood sugar was 135 mg. per cent. Electrolytes at the time of admission showed sodium to be 143.7; potassium, 316; chloride, 95; and CO_2, 31.3 mEq. per liter. Blood albumin was 5.8 gm. per cent; globulin, 0.6 per cent.

The bowel was decompressed by a Homer-Smather tube connected to Wangensteen suction. The decompression was prompt and, except for a temperature spike to 40°C. on the third day, the recovery was uneventful. Further laboratory tests showed serum non-protein nitrogen of 21 mg. per cent on March 15, and the white blood count was 11,200/mm.^{3} with 79 segmented forms, 7 stab forms and 14 lymphocytes. The electrocardiogram, which had been previously within normal limits, now showed a right bundle branch block. A chest roentgenogram revealed no abnormalities. Examination of the upper gastrointestinal tract revealed the first part of the duodenum to be displaced upward and the third part to be displaced a little to the right. The small bowel examination revealed an extrinsic mass 6 cm. in diameter in the right periumbilical region and some displacement of the stomach consistent with splenic enlargement. A barium enema revealed no abnormalities.

Repeat blood protein studies showed an albumin of 5.0 gm. per cent and a globulin of 1.0 gm. per cent. Paper electrophoresis of the serum revealed no gamma globulin. The patient's blood was type A, Rh negative, and there were no alpha or beta isoagglutinins demonstrated. Sedimentation rate was 1 mm. per hour. The heterophile test and agglutination tests against brucella, typhoid O and H, paratyphoid A and B, and tularemia all gave negative results.

The patient was discharged in satisfactory condition. Six months later he was found to have remained well and to have been working regularly.

COMMENTS

Some of the juvenile cases of agammaglobulinemia previously reviewed may represent a congenital defect in the formation of gamma globulin, possibly sex-linked in some cases, because of the predominant occurrence in males. Patient J. N., Case 1, no doubt acquired this disease in recent years, particularly since he suffered from asthma twenty years previously due to hypersensitivity to aspirin.

Speculation as to the pathogenesis of this disease introduces controversies over the cellular source of antibodies and gamma globulin. The evidence has recently been reviewed,[32–34] and points toward plasma cells or lymphocytes as the principal sites of antibody formation. The problem is complicated by varying opinions on the development and morphology of cells. Perhaps more than one cell type is concerned.

Using quantitative bone marrow counts of plasma cells, Good and Campbell[25] have been able to correlate plasmacytosis with the elevation of serum gamma globulin in patients with rheumatic fever. The low bone marrow plasma cell count found in these patients further suggests these cells as a source of gamma globulin.

The half-life of human gamma globulin has been established as twelve to fourteen days,[35] with a somewhat longer survival in children. The long half-life of passively transferred gamma globulin in these patients[1,3] suggests that the primary defect is deficient synthesis rather than excessive destruction of gamma globulin. Paper chromatography of amino acids was carried out in an attempt to discover some specific defect in the metabolism of amino acids but the amino acid spectrum was normal. However, the observation of abnormal amounts of conjugated ethanolamine in the plasma and urine of J. N. (Case 1) suggests further investigation of the role of this substance in gamma globulin formation. Conjugated ethanolamine has also been found in the urine of premature infants who are known to be deficient in antibody formation.[36]

Cohn et al.[37] have determined that most immune antibodies and isohemagglutinins reside in the gamma globulin fraction of plasma, while the major portion of C^1 and C^2 components of complement are contained in the alpha and beta fractions of plasma, respectively. The patients here described have no isohemagglutinins and were unable to produce any, whereas the serum complement was within normal limits. The presence of a normal serum complement titer in J. N. emphasizes the secondary role that complement plays in resistance to bacterial infection. It is ineffective as a bactericidal agent in the absence of specific antibodies.[38]

The etiology of the splenomegaly and the reason for the failure of J. N. to respond to infections with leukocytosis are obscure. At one time marked granulocytopenia was present. The condition of at least one pediatric patient with agammaglobulinemia has been complicated by granulocytopenia during infections; this patient was regarded for years as having recurrent splenic neutropenia before the agammaglobulinemia was discovered.[39] Menkin[40] has isolated a leukocytosis-promoting factor from inflammatory exudate and identified this as a pseudoglobulin distributed between the alpha-1 and alpha-2 globulins of exudate.[41] It is possible that some component of gamma globulin is necessary for the elaboration of this leukocytosis-promoting factor.

Although the recurrent respiratory infections and the skin rash, apparently infectious in origin, can be attributed to the agammaglobulinemia, it is more difficult to account for J. N.'s diarrhea. The flat glucose tolerance and vitamin A tolerance, the low-fasting carotene level, the slightly elevated stool fat on one occasion and the intensification of the diarrhea by high fat feedings all serve to classify this as a steatorrheal syndrome. The large nitrogen stool is compatible with this disease, according to recent studies.[42] Tumor was suggested by the transient intussusception, but the patients' subsequent improvement noted on roentgenograms and his clinical remission speak against this diagnosis. It is also possible that oxytetracycline was responsible, even with the delayed onset. Four patients with prolonged steatorrhea following the use of antibiotics have been reported,[43] and in one of these the onset began a week after discontinuing administration of the drug. However, our patient subsequently tolerated oxytetracycline without difficulty. A plausible diagnosis is an unusual form of diffuse jejunoileitis, with the course influenced by altered response to infection. This could account for various deficiencies in intestinal absorption.[44]

Patient D. S. was unique among all patients with agammaglobulinemia reported in adults and children in that he was relatively free of repeated infections. However, inflammatory lymph node enlargement probably contributed to the first and was in large part responsible for the second episode of intestinal obstruction, which responded to conservative management.

SUMMARY

Two case studies of adult white men with agammaglobulinemia and splenomegaly are reported. One has been subject to repeated bacterial infections of the skin and respiratory tract and has had several attacks of severe diarrhea. The other patient has had recurrent small bowel obstruction probably due to inflammatory lymph node enlargement, but has otherwise been virtually free of infection.

The occurrence of this syndrome in adults is discussed.

Acknowledgements: The authors wish to thank Dr. Harry Alexander for his help and criticism in treating patient J. N. (Case I) and in the preparation of this manuscript; Dr. Richard Weiss, who first treated the skin infections and referred patient J. N. to us; Dr. S. Howard Armstrong for the special protein studies and for his helpful advice; Dr. Keith S. Wilson who diagnosed the condition of patient D. S. (Case II) and permitted us to include him in this report; Dr. Albert Mendeloff for the carotene and vitamin A determinations; Dr. Sam Frankel for the amino acid paper chromatography, Dr. David Gitlin for the gamma globulin determination by the immunologic technic and Dr. Amoz Chernoff for the paper chromatography of serum proteins.

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Bronchiolitis Obliterans*

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An infrequently described lesion, bronchiolitis obliterans, would appear to be a rare entity. The distinctiveness of the lesion makes it unlikely that it would not be recognized. It is pertinent that LaDue,[1] making a specific search, found only one case in 42,038 consecutive autopsies covering a forty-two-year period. And yet Winternitz[2] consistently found cases among dogs exposed to chlorine, phosgene, chloropicrin and other war gases. It would be anticipated that cases in human beings would have been found in large numbers after the gassing attacks in World War I. This does not seem to have been borne out. Muntsch[3] mentions bronchiolitis obliterans among the residual pulmonary effects of World War I gas poisoning but gives no indication of its frequency. It seems to have been a relatively frequent complication of the influenza pandemic.[4,5]

Since the original description of the lesion by Lange[6] in 1901—all references prior to 1949 may be found in reviews by LaDue,[1] Blumgart and MacMahon,[7] and Amoroso and McNally[8]—the scattered reports seem to justify three distinct etiologic forms of the disease: (1) following inhalation of irritant substances, (2) as a complication of pulmonary infection, (3) unknown etiology.

Fumes of oxides of nitrogen are the more common[1,7,9] of the irritant substances cited in the literature as causes of bronchiolitis obliterans. They may arise from burning nitrocellulose film, explosives, breakage of carboys of nitric acid or other containers, and from other sources.[10] The composition of the fumes will vary but some generalizations can be made.[9,10] Nitric oxide, itself non-irritating, undergoes comparatively rapid oxidation to nitrogen dioxide. From the toxicologic standpoint, nitrogen dioxide is the most important of the oxides of nitrogen.[11] It readily polymerizes to nitrogen tetroxide, existing as a mixture of approximately 30 per cent NO_2 and 70 per cent N_2O_4 at body temperature. Each is readily soluble in water and forms nitric acid which causes the lesion. Hydrochloric acid has been reported as a cause and sulfuric as well as unspecified acid fumes have been cited in a few cases. Edens reported on two patients with a typical clinical picture of bronchiolitis obliterans following exposure to acid fumes and strong ammonia, respectively, each of whom recovered.[12] One case was reported as due to a cellulose-containing furniture stain but the description leaves one uncertain that bronchiolitis obliterans is truly represented.[13] Reference to chemical warfare agents has already been made. In general, these agents have been chlorinated compounds.

Pulmonary infection has been responsible for some of the reported cases. Blumgart[7] reported a case in a boy with scarlet fever followed by pertussis, and reference is made to another unpublished case in a boy following pertussis.† The lesion was also seen as a frequent component of influenza in the great pandemic of World War I. Winternitz[4] stated that twelve of his ninety-five autopsied cases showed organization of the bronchiolar or alveolar exudate. The case of Lerfer and Winkler[14] followed a grippe-like illness. In an exhaustive study Hübschmann discusses the lesion in influenza.[5] Other infectious origins are also cited. Beitzke[15] discussed bronchiolitis obliterans as a sequel of acute (capillary) bronchitis. As a component of organizing pneumonia the lesion is probably not unusual. In such instances, however, the phenomenon is essentially a local one. This also applies to instances in which foreign bodies are concerned.

Bronchiolitis obliterans, apparently due to iodized poppyseed oil, has been reported but in the instance cited evaluation was made difficult

† This case (Autopsy No. 5-33) is from a collection of Dr. J. R. M. Innes, obtained while he was at the Institute of Pathology, Munich, under Prof. Max Borst, 1932 to 1933. (Permission of Prof. W. Hueck, Pathologisches Institut der Universität, Munich, Germany.)

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by a long standing history of respiratory disease prior to bronchography.[1] There are certain objections concerning a report of bronchiolitis obliterans associated with pulmonary vasculitis. Two of the three cases were in individuals with chronic cardiovascular disease. Several illustrations used to demonstrate vascular lesions, moreover, show altered bronchial arteries. Actually, the changes pictured are commonly noted lesions of the bronchial artery and have been specifically discussed by Liebow et al.[16]

The clinical picture[1,7,9,10,14,17] of bronchiolitis obliterans is variable at its inception, depending on its etiology. In the more common instances due to oxides of nitrogen or other irritant fumes, there is an initial period of cough, dyspnea, slight or severe chest pain and cyanosis. The patient is usually afebrile. Most fatalities occurring at this time show acute necrotizing lesions of the tracheobronchial tree and massive pulmonary edema. When pulmonary infection is the initial lesion, the symptoms correspond. There is then an interval of apparent improvement over a period of several days to a month or more, although a few cases show gradual progression. This interval tends to be short in cases due to inhalation of irritants.[18] The third stage appears to have the same clinical characteristics in all groups and is remarkable for its unremitting nature. The cases of uncertain etiology are generally first recognized in this stage.[19] Dyspnea becomes progressively and rapidly more marked, there is variable cough and expectoration of blood-stained sputum. Cyanosis is always conspicuous. There may be fever but this is generally low grade. In this third stage the roentgenogram of the chest may simulate miliary tuberculosis which may offer the main problem in differential diagnosis. The importance of this roentgenographic pattern in a patient with marked dyspnea is to be emphasized. It would be desirable to anticipate the disease before it develops but there are apparently no reports specifically demonstrating the evolution of the roentgen pattern. There are, however, reports of the changes in x-ray in acute poisoning due to oxides of nitrogen which can be taken to represent the probable early pattern of those going on to develop bronchiolitis obliterans. Soon after exposure there is an irregular, soft mottling throughout both lung fields. These patches of increased density follow the course of the bronchial tree and extend to the periphery of the lung fields,[18,20] tending to be more numerous in the mid-lung fields.[15] There is then confluence of minute areas of atelectasis and edema, with development of larger nodules. This stage is remarkable for the rapidity with which the chest film clears in those who recover.[20–22] However, the miliary-like densities may persist. In this regard Nichols'[23] experience with the Cleveland disaster (1929) from burning nitrocellulose film is of some interest. He found that while the clinical examination of the chest was often unremarkable, the roentgenogram always revealed many small nodular areas scattered throughout each lung. On the basis of preliminary autopsy findings in fatal cases, these densities were explained as being due to foci of interstitial fibrosis. However, the fact that a group of the victims showed a phase of clinical improvement followed by relapse with severe cyanosis approximately fifteen days subsequent to the exposure is strongly suggestive that these foci of "interstitial fibrosis" were actually the lesions of bronchiolitis obliterans.

Pathologically, the appearance of the gross lesion is distinctive and, if the clinical picture is known, should not be confused with miliary tuberculosis which it closely resembles.[1,7,8] The parenchyma of all lobes is studded with small nodules, generally 1 to 2 mm. in diameter, which are serrated in outline. Careful observation of the cut surface of the nodules reveals a slit-like or rounded space in the central portion of many, the remnant of the bronchiolar lumen. The latter feature is important in differentiation from miliary tubercles. Miliary lesions in other body locations are absent. Emphysema and atelectasis, singly or in combination, may be found and the lungs are frequently edematous and show focal hemorrhages. Such hemorrhage was a conspicuous feature in the experimental lesions produced by Winternitz[2] and could be correlated with the violence of the agonal state.

The descriptions of the histologic changes of the several reported cases vary, even in cases due to the same cause. It is possible that this reflects more than one mechanism in the pathogenesis of the lesion. In the characteristic lesion[1,7,15] the lumen of the bronchiole may be partially obliterated by granulation tissue. The obstructing mass often has a polypoid appearance and is separated by a space from a crescent of intact, probably regenerated, bronchiolar epithelium, or the lumen may be entirely obliterated by young connective tissue. There

is a diversity of opinion concerning the earlier stages which end in this picture. The earliest lesion is certainly an acute bronchiolitis with destruction of the epithelial lining. When irritant fumes are the etiologic factors there is probably accompanying alveolar injury. This is based on the observation that most severely exposed individuals die early with massive pulmonary edema. Extensive necrosis of the wall of the bronchiole apparently does not generally occur in these patients.[1,4,7,12] In this respect it is significant that bronchiolectasis is infrequently noted.[1] That the damage may be more than simple inflammation of the bronchiolar wall is implied by most authors who describe disruption of the muscularis and elastica. The source of the granulation tissue is generally agreed to be the wall of the bronchiole, and it is here that there is the most divergence of opinion, i.e., whether it arises in the submucosa or muscularis, represents an infiltration of peribronchiolar origin, or is a reaction of the wall as a whole.[24] Involvement of neighboring alveoli is mentioned in most reports, and it is improbable that there have been any cases in which contiguous alveolar structures were not involved. This is certainly so in cases following influenza, as pointed out by Winternitz.[4] Most authors consider alveolar involvement to be secondary to the bronchiolar involvement. However, an alveolar origin of the process leading to bronchiolitis obliterans has not been convincingly excluded.[18] Groll's excellent description clearly demonstrates the role of alveoli in the organizing process.[25]

CASE REPORT*

On December 29, 1953, the patient, a thirty-eight year old chemist employed by the U. S. Bureau of Mines, aided in the rescue of a colleague after a group of flasks containing red fuming nitric acid exploded as they were being vented. The laboratory was quickly filled with dense fumes, chiefly of nitrogen dioxide. There is little information about early symptoms other than that the patient is said to have had a cough following the accident, and had tightness in the chest the first few days. He took sick leave, unusual for him, but complained of no symptoms other than cough until January 19, 1954. The cough increased in severity and he experienced chilly sensations with fever. He consulted a physician for the first time on January 21, 1954. Only loud rhonchi were noted on examination of the chest. His symptoms became rapidly and progressively more marked, and retrosternal pain, a feeling of tightness in the chest, and later shortness of breath developed. Treatment with aureomycin was begun on January 23, 1954. The following day shortness of breath was so severe that he had to remain in a sitting position.

On admission to the hospital on January 24th, he had a dry hacking cough, dyspnea showing marked respiratory effort and a respiratory rate of 52/min. Temperature was 100.4°F., pulse 128/min., blood pressure 170.90 mm. Hg. Abnormal findings, limited to the chest, were wheezing, sonorous breath sounds and râles throughout. The urine was normal, the red blood count was 4.05 M., white blood count 11.1 T., hemoglobin 13.1 gm. per cent and a differential count of polymorphonuclear leukocytes 81 per cent; lymphocytes 14 per cent, stab forms 3 per cent and eosinophils 2 per cent. A posteroanterior chest film on admission revealed small, irregular densities, 1 to 2 mm. in diameter, diffusely scattered throughout the lung fields. These miliary-like densities had indistinct, irregular margins and were of uniform radiopacity. There was a closer relation to bronchial markings than to vascular ones. (Fig. 1.) The patient was given aureomycin, intravenous dextrose and saline. His temperature fell to 99.4°F. and he remained afebrile. The hospital course was one of rapid deterioration. Cyanosis of the mucous membranes and nail beds was noted and progressively deepened. Respirations gradually became shallow, the blood pressure fell and the patient expired on January 25, 1954, approximately thirty hours after admission.

At autopsy the significant findings were limited to the chest. The tracheobronchial tree was noted to be injected throughout and contained tenacious, purulent material. The lungs were markedly edematous and nodular on palpation. The cut surface of the formalin-fixed tissue blocks obtained from the Medical Examiner revealed the character of these nodules. (Fig. 2.) Most were from 1 to 2 mm. in

* The existence of this case was called to the attention of Chemical Corps Medical Laboratories after the autopsy had been performed. Material for examination was obtained from the Chief Medical Examiner, State of Maryland, and additional slides were obtained from the Armed Forces Institute of Pathology. Clinical data and x-ray films were supplied by the Prince Georges General Hospital, Maryland.

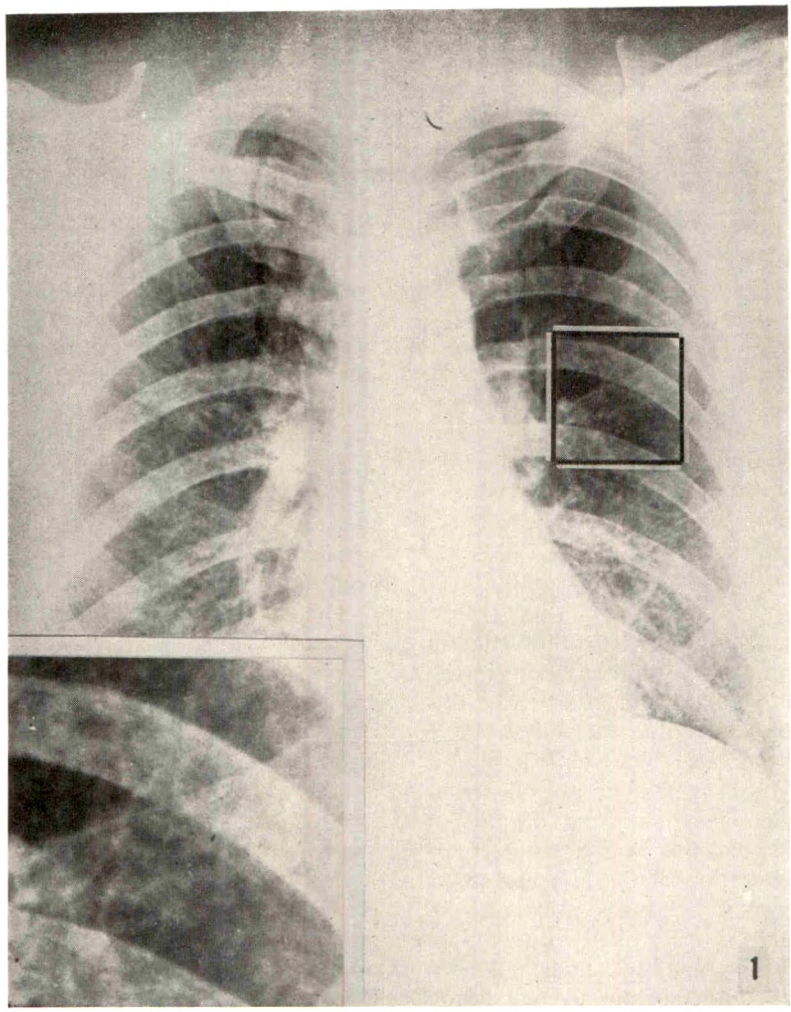


FIG. 1. Postero-anterior x-ray of the chest taken on January 24, 1954. Miliary-like densities may be seen diffusely scattered throughout both lung fields. The inset shows the details of the densities in their normal dimensions.

greatest dimension although occasional ones were somewhat larger. Their margins were so irregular as to be described as stellate. In the central portions of many were slit-like or round spaces representing bronchiolar lumens.

Histologically, all sections of the lung showed marked edema and many focal areas of hemorrhage. The characteristic lesion noted involved the bronchioles. (Fig. 3.) The lumen was virtually obliterated by organizing exudate which often projected in a polypoid fashion. Generally, a slit-like space separated the mass from a crescent of bronchiolar epithelium, in part showing hypertrophy of epithelial cells. Squamous metaplasia was not noted at this level of the bronchial tree. Within the organizing mass there was dense proteinaceous material and varying inflammatory cell infiltration. Although this proteinaceous material had a fibrinoid appearance, it was not characteristically stained with phosphotungstic acid-hematoxylin. Lymphocytes and monocytes were the predominant cells although polymorphonuclear leukocytes were also noted. Eosinophils were not conspicuous. The invading fibrous tissue was highly vascular, appearing as a delicate stroma with little collagen. The submucosa of the bronchiole was greatly swollen and infiltrated by inflammatory cells, lymphocytes being more prominent than polymorphonuclear leukocytes. There did not appear to be a significant degree of fibrosis of the submucosa. The muscular wall was essentially intact. The bronchial tree at this level is a multibranched structure with a muscularis

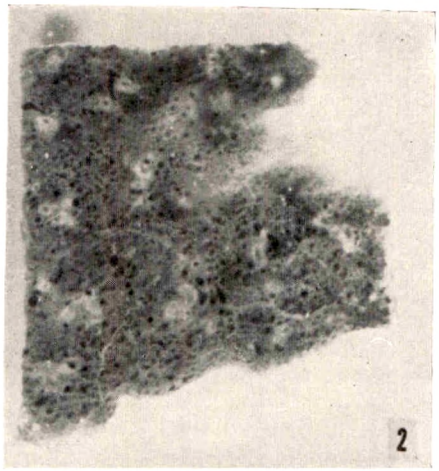


FIG. 2. The cut surface of a formalin-fixed block showing several of the nodules. Note that in many the bronchiolar lumen can be identified. × 2.

consisting of bands of muscle rather than a continuous sheath. Normal interruptions in the continuity of the wall therefore had to be differentiated from defects due to a destructive process. The bronchiole was cuffed almost exclusively by lymphocytes. The alveoli neighboring the bronchiole showed an organizing pneumonitis. In part this was relatively advanced with obliteration of the alveolar structures but more acute changes were also in evidence, some alveoli being filled with exudate composed chiefly of polymorphonuclear leukocytes. "Epithelialization" of the alveolar lining was commonly noted. There was no apparent alteration of the original lumen diameter of the bronchiole, as judged by the outline of the muscular wall. Bronchiolectatic lesions were conspicuous by their absence.

In none of the many sections examined were completely normal bronchiolar structures found. The minimally involved bronchiole (Fig. 4) possessed a completely intact and essentially normal mucosal epithelium. The submucosa, however, was moderately swollen and infiltrated by both lymphocytes and polymorphonuclear leukocytes. The muscularis was uninvolved but there was slight peribronchiolar scarring and infiltration by lymphocytes. Somewhat more advanced lesions were noted, still with the mucosa intact, in which the lumen was filled with exudate. Only when there were breaks in the continuity of the epithelial lining were masses of dense proteinaceous material present, and only in this circumstance did organization of the exudate occur. (Fig. 5.)

There appear to be two ways in which the exudate in the lumen of the bronchiole can become organized. On the one hand, this process appears to take place exclusively within the bronchiole. (Fig. 6.) Here one can readily make out the proliferating fibrous tissue arising in the granulating base of the submucosa. At least as often, however, this process of organization appears to be the result of invasion by the reactive process in the contiguous alveolar ducts and alveoli (Fig. 7), fibroblastic tissue arising from the organizing alveolitis invading the exudate within the bronchiole. Since alveoli arise from the respiratory bronchiole and alveolar duct as well as from the atrium and sacculus alveolaris, it is significant that the respiratory bronchiole seems to be the site of greatest organization.

The wall of the bronchiole remains essentially intact. (Fig. 8.) The elastica and muscularis showed no significant defects, the only interruptions being those due to the branching character of the structure. In the most advanced lesion the bronchiole was completely obliterated, as illustrated in Figure 9. In this instance, involving a respiratory bronchiole, the continuity of the organizing process within the lumen and of the process involving the surrounding alveolar structures is clearly evident. At the more peripheral locations in the lung it is often difficult to distinguish much more than a focal area of organization, as shown in Figure 10. There appear, however, to be some bands of smooth muscle even here. The focal nature of the lesion is probably significant. The entire tracheobronchial tree was evidently involved at some stage because foci of regenerated epithelium in the trachea still retain some characteristics of squamous epithelium, as illustrated in Figure 11.

COMMENTS

Careful consideration should be given to the use of the term bronchiolitis obliterans. Ehrich and McIntosh[18] maintained that the designation is justified only when the process is widespread. That similar lesions occur as focal phenomena in many organizing processes is well known; in fact, such organization may be widely disseminated. It is significant that these are often purely morphologic findings without clinical correlation and frequently not obliterating lesions at

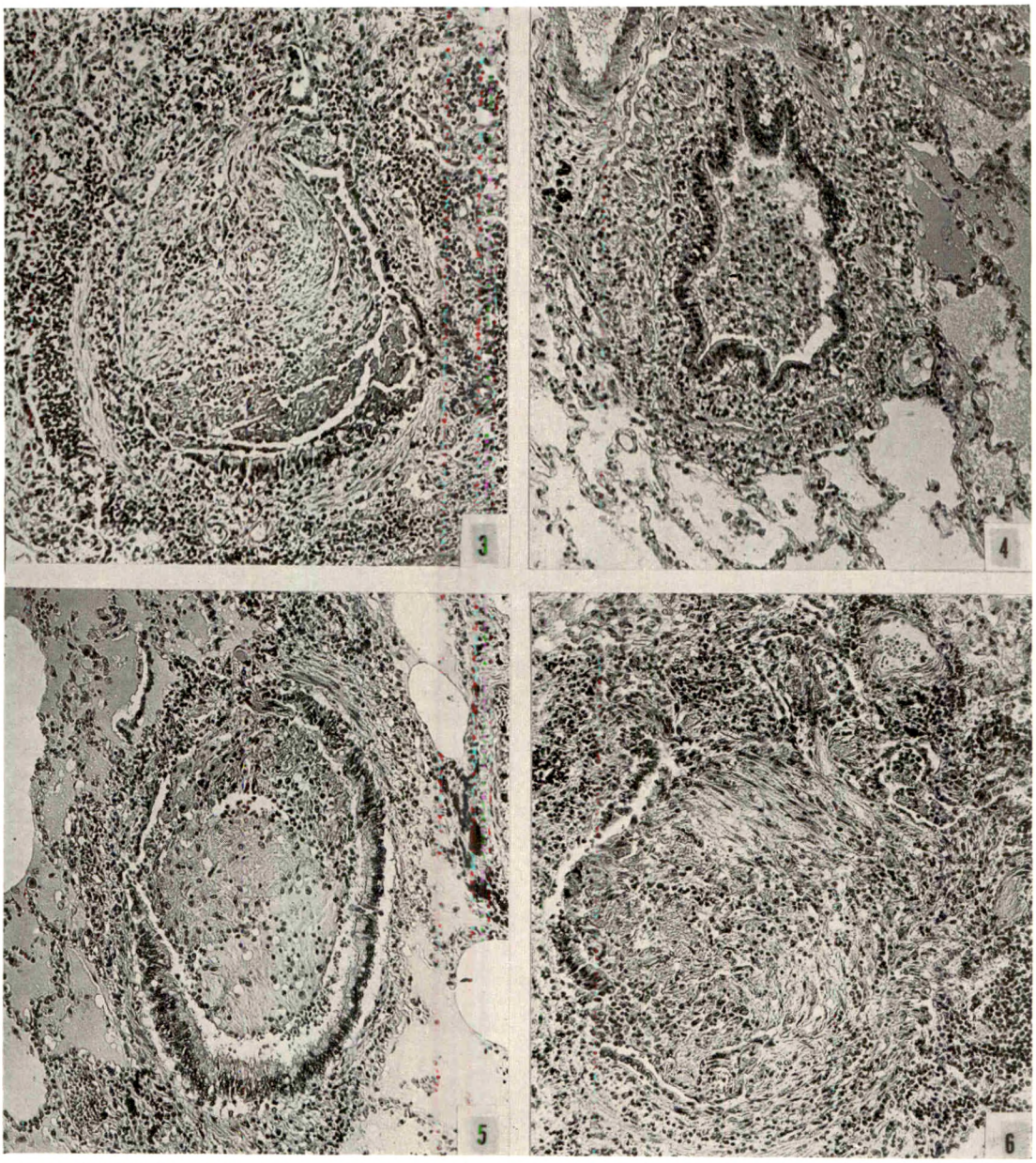


FIG. 3. The typical lesion of bronchiolitis obliterans showing the polypoid appearance of the occluding mass; hematoxylin and eosin stain.

FIG. 4. A minimally involved bronchiole. Possibly, a more distal section of this bronchiole would show involvement by the obliterating lesion; hematoxylin and eosin stain.

FIG. 5. Proteinaceous material in association with a defect of the bronchiolar mucosa; hematoxylin and eosin stain.

FIG. 6. Granulation tissue arising from the wall of the bronchiole and occluding the lumen; hematoxylin and eosin stain.

all but lesions of early fibroblastic proliferation. This objection might be raised with reference to Ehrich and McIntosh's three cases, all of uremic pneumonitis in which such phenomena as fibroblastic proliferation are extremely common. Considering the reserve ventilatory capacity of the lung, disseminated lesions would not be expected to produce the clinical picture seen with lesions of a generalized nature. Perhaps it would not be superfluous to speak of bronchio-



FIG. 7. Exudate in a bronchiole showing organization arising from the plastic process in contiguous alveoli; hematoxylin and eosin, × 115.

litis obliterans generalisata. The clinical picture is unique so that any purported case not accompanied by such features should be suspect.

There are additional facets of the case reported herein which are noteworthy. With respect to the question of the origin of the newly proliferated connective tissue, it is not surprising that a dual source was found: viz., from the granulating submucosa of the bronchiolar structure itself and as an ingrowth from alveolar structures. This is contrary to the belief of most authors, the general impression being that alveolar involvement is secondary. That alveoli may suffer primary damage is unquestionable where irritant fumes are concerned, and this is clearly demonstrated by Winternitz et al.[2] in their studies on war gas poisoning. Figure 12 is instructive in this respect, the section being that of lung from a dog exposed to red fuming nitric acid fumes at 75 ppm for five hours and dying five and a half hours from the time of onset of exposure. It shows unmistakable damage to the bronchiole and

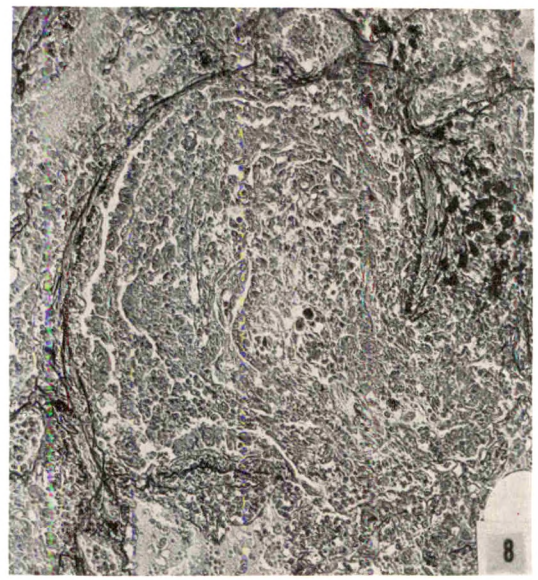


FIG. 8. A typical lesion of a bronchiole with normal elastica and muscularis; elastica, × 115.

alveolar duct, as well as necrosis of the alveolar septal wall. Such findings argue against the view that involvement of the bronchiole, short of the alveoli, is due to bronchoconstriction.[19] The dog is unique among mammals in respect to the degree to which the musculature of the respiratory bronchiole and alveolar duct is developed, and this might be correlated with isolated circumstances under which there are very marked increases in bronchial resistance. A more simple explanation of the level at which injury is maximum is based on the solubility of the material in question. Hydrochloric acid is more soluble in water than the oxides of nitrogen, and therefore the effects of hydrochloric acid are maximal in the upper airway, whereas those of the oxides of nitrogen are more deeply located.

There seems to be a segmental distribution of the lesions, for the total lesion is relatively uniform in size and considerably smaller in area than would correspond to a group of alveoli arising from a single bronchiole. Larger lesions seem to be predisposed to complete obliteration. The respiratory bronchioles and alveolar ducts appear to be the sites of maximal organization, and alveolar involvement predominantly affects alveoli arising direct from the respiratory bronchiole and alveolar duct.

From a nosologic standpoint, it is evident that bronchiolitis obliterans can be considered only when the full blown clinical as well as the

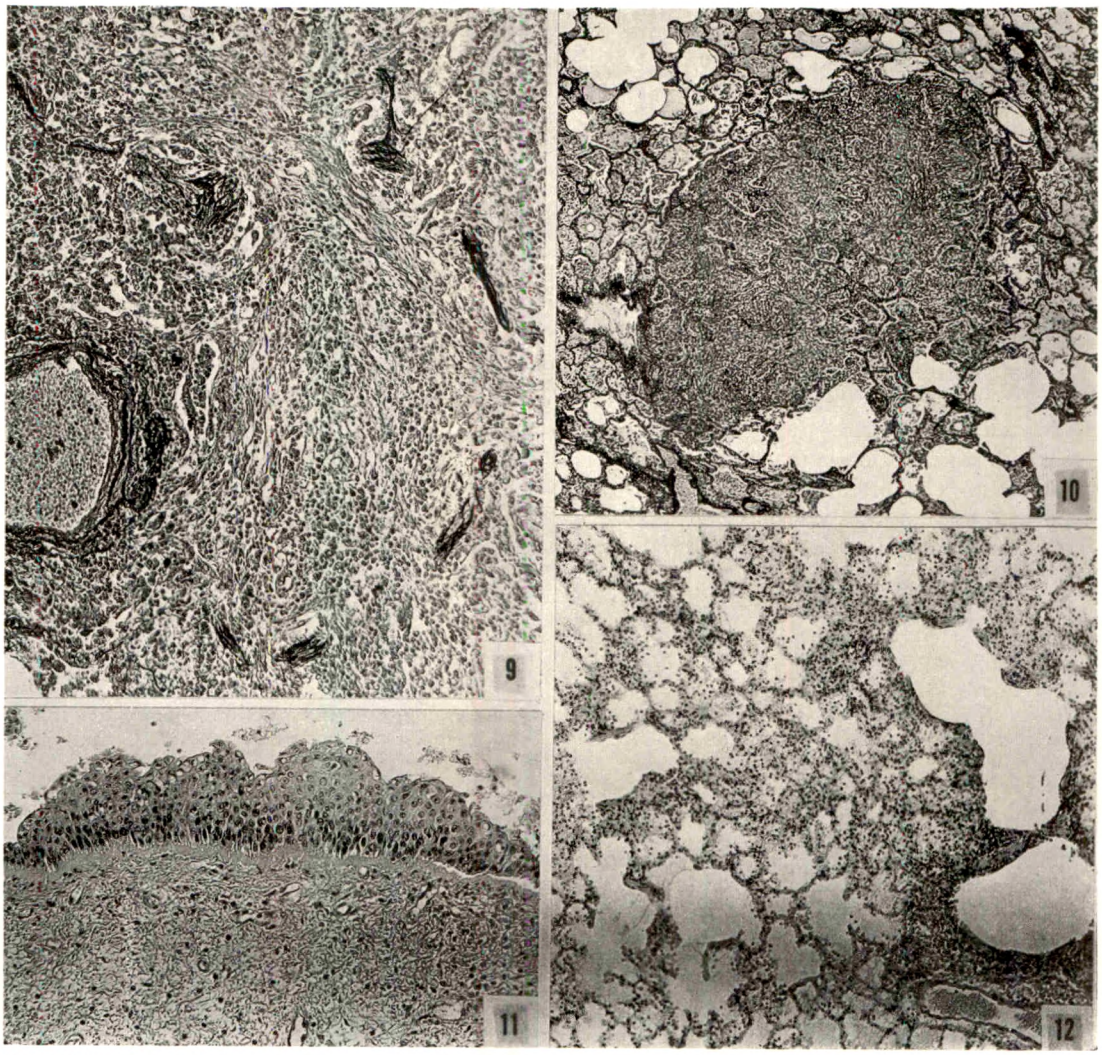


FIG. 9. Complete obliteration of a respiratory bronchiole showing continuity of the granulation tissue in the alveoli and bronchiolar lumen; elastica stain.

FIG. 10. A nodular area of organization in the periphery of the lung; elastica stain.

FIG. 11. An area of squamous metaplasia in the mucosa of the trachea; hematoxylin and eosin stain.

FIG. 12. Lung of dog exposed to red fuming nitric acid fumes. Note the necrotizing lesion of the respiratory bronchiole and alveolar duct as well as the focal alveolar necrosis; hematoxylin and eosin stain.

pathologic picture is present. The lucid description by Beitzke[15] of the manner in which bronchiolitis obliterans of infectious origin develops makes it clear that, apart from a greater inflammatory component, there is marked similarity with that due to chemical injury.

Poisoning by inhalation of irritant substances, in particular fumes of oxides of nitrogen, is by no means uncommon and in the past has accounted for the larger number of cases of bronchiolitis obliterans. Acute (pulmonary edema) rather than delayed fatalities are the rule, however.[3,9,10,23,26] Nitric acid, particularly red fuming and white fuming, is an extremely important substance in certain industries and today assumes added importance in its use as an oxidizer in rocket fuel. With the continued increase in the use of such substances, it is to be expected that there will be increasing numbers of similar industrial accidents. Physicians treating such patients should be alerted to this disease as a late complication. Furthermore, the increas-

ing effectiveness of treatment in the acute phase may mean that more patients will survive only to succumb at a later date.

SUMMARY

Bronchiolitis obliterans should be classified with the diseases of unremitting dyspnea. The clinical picture is distinctive and correlates with the generalized involvement of respiratory bronchioles by obstructing lesions. Of particular importance in diagnosis is a roentgen pattern simulating miliary tuberculosis.

Pulmonary infection or the inhalation of irritant fumes are the most common causes. The case reported herein was due to inhalation of nitrogen dioxide, which is the single most common cause of bronchiolitis obliterans. Attention is drawn to the fact that poisoning by oxides of nitrogen fumes is not at all uncommon.

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The American Journal of Medicine

VOL. XIX SEPTEMBER, 1955 NO. 3

Editorial

"For Breath Too Is Nutriment"

WHEN we treat a patient with chronic "non-specific" pulmonary disease we often feel ourselves in a trackless quagmire of contradictory recommendations for therapy in a condition which we are ill equipped to diagnose and which, by its bewildering complexity of seemingly unconnected manifestations, paralyzes our powers. All around us, among our professional colleagues, in the current medical literature, in hospital charts and laboratory reports there is an incomprehensible chatter, a flowing alphabet soup of cryptic and code-like jargon, foreign to our ear, of M. B. C. and F. R. C. interspersed all too frequently with an algebraic expression or, worst of all, with that Indian sign to the medical mind, a differential or an integral symbol.

A congeries of factors seems to have thrust us into this slough: here are bronchodilators, there are emphysema belts, yonder aerosol generators (perhaps with design to insufflate or exsufflate), everywhere are antibiotics in bewildering profusion which might cure our patient's infection or, who knows, might turn the bronchial passages into a defenseless playground for an overgrowth of ordinarily innocuous but now slimily coalescent pathogens raised like an army of zombies from the normal microbial flora. And down the hall dwells a young scientist with burning eyes and glistening spirometers who, facile in crypto-alphabetese, would, at the twist of a stopcock, turn emphysema into fibrosis while threading a heart with nylon tubes. Not the least of our reactions is guilt. We feel we should know, that others do know, the solid from the quicksand in this swamp, and boundless anxiety issues from our neurotic guilt.

There is some justice in our view. The mire of chronic non-specific pulmonary disease is not new, and the weirdly festooned and clustered woods of pulmonary impairment have always been; but as gloom dispels in the growing light of medical science we can see the situation more vividly if not more clearly. Meanwhile the proportion between middle and older aged in the population is continually increasing, the killing diseases of childhood and youth are decreasing, so that the relative incidence and importance of the insidiously progressive chronic non-specific pulmonary diseases are multiplying. Some confusions concerning the origin of dyspnea have been resolved. In many instances the heart may be exonerated and the lungs indicted. If we erroneously diagnose failure of the former when the latter is the culprit, time will confirm us in our error because heart failure will inevitably occur as a result of pulmonary failure, if something else does not carry our patient off in the meantime. Finally, effective diagnostic and therapeutic measures have come much more plentifully to our hands. That a situation is susceptible of management imposes upon us a duty to recognize and to treat it skillfully.

Much of our self-recognized befuddlement is derived from the fact that at the time we attended medical school chronic pulmonary insufficiency was a troublesome, refractory and fortunately (in a sense) an infrequently recognized condition receiving minimal curricular attention. We feel the lack of systematic grounding in the basic principles and now, harried, we are attempting to fill the hiatus piecemeal. Perhaps it would be comforting to look to the past to see how long a path has really been

traversed and how slowly we have moved along it.

A place to start might be that " . . . earliest surviving example of observation and conclusion, the oldest known evidence of an inductive process in the history of the human mind," the Edwin Smith surgical papyrus (Breasted). This document, which dates from the sixteenth century B.C., copies a much older text. The latter, about the twenty-sixth century B.C., was in itself an annotated version of one originating, it is thought, in the Pyramid Age around the thirtieth century B.C. The instructive form directs us to examine the patient and conclude one of the following: "an ailment which I will treat"; "an ailment with which I will contend" or "an ailment not to be treated." Chronic pulmonary disease of the sort which concerns us never falls in the first category of curable illness and should not be placed in the third of hopeless illness. Chronic disabling pulmonary disease is an ailment with which the physician must "contend."

To do this we must know the natural history of the disorders we propose to manage "I hold it is an excellent thing for a physician to practice forecasting," said Hippocrates (*Prognostica*) "for if he discover and declare unaided by the side of his patients the present, the past and the future and fill in the gaps in the account given by the sick, he will be the more believed to understand the cases, so that men will confidently entrust themselves to him for treatment. Furthermore, he will carry out the treatment best if he know beforehand from the present symptoms what will take place later. . . . " Hippocrates, if he were about, would nowadays be willing to modify "unaided" to encourage recourse to the needed specialists in radiology, electrocardiography pulmonary function, bacteriology and cardiac catheterization, but would doubtless sternly insist that we remain at the patient's side to convert the specialists' information to useful plans for the patient. This we can do only through a working comprehension of these ramifications of the modern art acquired from study, if not from practice, of their technics.

Anaximenes of Miletos proposed air, pneuma, as the primary substance " . . . permeating everything" and indeed, from the biologic point of view, such is true. Every part and system of the body is permeated with air and dependent upon an endlessly refreshed supply of it for well-being. Interference with the bodily aerating equipment must of necessity manifest itself in myriad ways.

Complexity, however, cannot cause mental panic unless we allow it. A child of seven steps up to a television set and unhesitatingly tunes in a station, adjusts the horizontal and vertical raster control circuits to the locking signals of the transmitting station, focuses the electronic beam unerringly, adjusts the bias of the control grid of the cathode ray tube to a degree of apparent contrast to suit his fancy, turns the audio volume control wide open and thunders off into outer space in a reaction-engined craft without a qualm. Confronted with a circuit diagram of the set, he would blink with incomprehension, a reaction that will persist with him well into engineering school a decade and more later. There he will be taught an engineer's trick: reduce the incomprehensible whole into comprehensible parts. Here is the power supply, here its filter network, here the automatic volume control, and so on. Thus, piece by piece, the whole becomes simple. There is no scientific concept borne of the human mind which is not simple in its basic elements. To get at these basic elements may be another matter! When the work is new it is difficult to thread a way among the conflicting data, but with time and the work of many minds the findings are digested, condensed, sorted and simplified to a teachable or learnable unit of knowledge. How far we go in our studies of this subject is a purely personal matter but whatever we attempt all will be lost if we swamp ourselves in complexity instead of systematizing our attack on the problem and subdividing it into comprehensible elements. This is difficult if not impossible to do alone in a field where areas of comprehension are chequered among larger areas of ignorance.

Engineers can teach us another trick for working with systems we do not or cannot fully understand: the useful tool of the four terminal network parameters. Here an electronic device is envisaged as though two wires, the input circuit, disappeared through two terminals into a "little black box," and two wires, the output circuit, emerge through two other terminals from the opposite side. It is often entirely unnecessary to know the contents of the box; it may be enough to know what changes are produced in the output circuit by certain changes in input, and vice versa. A "little black box" can be made to contain that part of any system about which the technical details

are unknown, or as much or as little as desired. So if for a given amount of power fed into the "little black box" a lesser amount emerges, it is evident the loss occurred within the box, while if there is gain of power it is axiomatic that the box is playing an active role and its behavior in this respect could be characterized by a generator of appropriate properties. In either event we would want to know where the lost electricity went or where the gained energy originated, but these are questions for the research laboratory.

The "little black box" will serve very well for the bedside. It has to be accepted definitely that we cannot understand everything. As researchers open one by one the "little black boxes" and show their contents to us, more and more unopened boxes are revealed. It is poor research indeed which does not raise more ghosts than it lays. We may conveniently place such matters which we do not understand into useful "little black boxes." Thus we can make intelligent use of the knowledge that the volume of air moved by the bellows action of the lung during maximal voluntary hyperventilation increased 20 per cent following administration to the patient of a bronchodilator drug to lead us to the conclusion that bronchoconstriction is a factor in our patient's malady despite a limited comprehension of all the complex mechanisms involved. Such reasoning should not be adduced as an excuse for unnecessary ignorance but rather as a working technic with what knowledge has been allowed us.

Empedocles of Agrigentum, he of the four elements, fire, air, water and earth, who flourished early in the fifth century B.C., performed a crucial experiment which proved the corporeality of air. By plugging the hole in one end of a clepsydra (a hollow vessel with holes at each end) with his finger and immersing the vessel in water he showed that the entrapped air prevented the entrance of water until he removed his finger from the top hole and let the air escape. Not much of vital interest to physiologists was added to this information about air until 1668 A.D., some twenty-one centuries later.

The Hippocratic physicians were close observers of the respiratory movements. Thus the second case described in *Epidemics* I: "from the beginning the breath in this case was throughout rare and large." Hyperpnea, we may suppose, since the clinical picture described is dehydration and metabolic acidosis in a patient dying of acute fever with copious diarrhea. Again, Cheyne (1818) and Stokes (1846) were antedated in their observations of the kind of breathing which commonly bears their names by the author of Case 15 in *Epidemics* III: "Respirations rare and large with long intervals becoming afterwards short."

The forty-eighth chapter of *De Alimento*, an aphoristic work of the Hippocratic corpus of uncertain authorship written about the turn of the fifth century B.C., which is taken as the text for this editorial, shows the preoccupation of this school with the way of breathing and the "obscure sybylline" utterances they were forced to take refuge in because of the lack of real comprehension of respiration. To recognize air as a "food," Sarton points out, could only then have been a guess or a metaphor. The point was not proved until Priestley in 1772 rediscovered Mayow's "igneo-aerial" spirit which he isolated as oxygen, and Lavoisier soon afterwards disposed of the phlogiston theory setting the matter straight for all time.

Bewilderment about the purpose of respiration persisted for centuries despite the fact that it was known to the earliest Egyptians, even to their paleolithic predecessors, that air was essential to life. Murder by suffocation is not a new invention, and execution by entombment (and consequent suffocation) did not begin with Aida. Jelliffe traces the more recent developments: Galen thought respiration was for the purpose of cooling the heart and this doctrine persisted until recent times. Harvey recognized the existence of "two different currents" of the blood but supposed one to generate the vital spirit in the left side of the heart, the other to get rid of the foul vapors and the heat of the innate fire burning in the heart. Von Helmont recognized the existence of "fixed air" (carbon dioxide) from his studies on fermentation but had no idea what it was, and the concept was lost. Borelli elaborated upon the concept of the bellows action of the chest and with others through the seventeenth century clarified the mechanics of respiration, coupling in the observations of Malpighi on the minute structure of the lung and advancing finally to the point of recognizing that the "particles" of air enter into and become mixed with the blood. Hooke also proved the necessity of air for life by experiments in artificial respiration, and Lower "by his careful transfusion experiments demonstrated that the difference between dark venous blood and lighter arterial blood was due to the admix-

ture of air thus explaining the color change which had been recorded for thousands of years and referred to thousands of causes."

John Mayow in 1668 cracked the problem left for him by Empedocles two thousand years before proving that air, while indeed it had corporeality, was not a single substance.* "Not all of the air was used by the lungs in influencing the blood but only a certain part of it, and although he called it by a different name (*spiritus nitro-aerius*) it was that part now known as oxygen." He saw that respiration was a process " . . . analogous to combustion, that both fire and breathing removed from air the *igneoaerial* particles" (Foster). Stephen Hales first stated the principle of free and combined gases and Joseph Black rediscovered Von Helmont's "fixed air," the carbon dioxide given off by the lung. When, after a hundred years of forgetfulness of Mayow's work, Priestley and Lavoisier, as earlier mentioned, elaborated upon the elements of external gaseous respiration, it remained only for Gustavus Magnus, just a century ago, to prove the presence of the gases in different proportions in the blood and thus complete "the last chapter in the unraveling of this secret of nature." The stage was set for the great advances in pulmonary physiology, especially in its quantitative aspects, made over the last century.

* Some credit Robert Boyle or Robert Hooke with this discovery.

Unfortunately the original literature on chronic pulmonary disease is enormous. In one segment of the field alone, pulmonary function testing, over seven hundred authors during the last two decades have published articles which have attracted enough attention to be quoted one or more times in other papers. The leading articles have been disseminated for the most part among forty-three different journals without much concentration in any particular one. Just in the last two decades have reviews, texts and, most useful, organized postgraduate courses on the subject begun to appear. Widespread use of Roentgen diagnosis, the advent of practical tests of pulmonary function, the ready availability of effective antibiotics and effective therapeutic products of synthetic chemistry, particularly bronchodilators, all within our clinical lifetime is bringing us nearer comprehension of that mystical but inspired passage: "Pulsations of veins and breathing of the lungs according to age, harmonious and unharmonious, signs of disease and of health, and of health more than disease, and of disease more than health. For breath too is nutriment."

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Clinical Studies

The Houssay Phenomenon in Man*

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RECENTLY two patients with diabetes mellitus have been encountered in whom there was rapid amelioration of diabetes, and insulin administration was discontinued. One patient, still alive, has generalized sarcoidosis, and signs of pituitary insufficiency appeared concomitantly with disappearance of the signs of diabetes; the other patient died suddenly and an abscess of the pituitary gland was found at autopsy. Another case of "disappearing" diabetes, in association with infarction of the pituitary, was brought to our attention by Dr. Frank Ford. Since individuals with panhypopituitarism can now be returned to a more or less normal state of health by judicious hormonal replacement therapy,[1] it is imperative to recognize pituitary insufficiency when it occurs. If insulin administration be continued in the diabetic who develops panhypopituitarism, severe hypoglycemia may occur and death may follow quickly; but if the panhypopituitarism be recognized, proper treatment allows continued life. Pituitary insufficiency should be considered immediately in the diabetic if the requirement for insulin decreases very rapidly, or if insulin shock occurs with great frequency and hypoglycemia is prolonged and relatively resistant to the administration of intravenous glucose.

The three cases to be described are instances of such an occurrence.

CASE REPORTS

CASE 1. P. H. (Johns Hopkins Hospital No. 160105), a twenty-two year old white diabetic, was in the ninth month of her first pregnancy when she entered the hospital on the Obstetrical Service in December, 1938. She had had headaches of progressive severity for one month, frequent insulin shocks and edema of the legs. There was no familial history of diabetes. Diabetes developed when the patient was thirteen years old, and because of its severity 70 units of crystalline insulin daily had been prescribed by her physician in addition to dietary regulation. Dietary indiscretions were frequent, as well as omissions of insulin administration; the patient was treated for severe diabetic acidosis on several occasions. Frequently she had episodes of insulin shock when she omitted one or two meals. There were always promptly relieved by administration of glucose intravenously. During pregnancy, the diet was largely uncontrolled and the diabetes was managed by her physician only with great difficulty.

Five weeks before admission malaise developed followed in a week by the sudden onset of severe headaches, anorexia and generalized weakness. Generalized anasarca then developed. During this time insulin shock occurred often and usually at night. In the attacks occurring during the week before admission the patient was observed to have generalized convulsions. Insulin dosage was reduced but hypoglycemic episodes occurred. On admission she appeared ill. There was tachycardia and tachypnea. She was weak and drowsy. There was generalized edema. Flame-shaped hemorrhages were seen in the left retina. Blood pressure was 112/70 mm. Hg. in the recumbent position. A viable infant estimated to weigh 2,600 gm. could be palpated on abdominal examination.

The patient was kept at bed rest. Despite careful dietary control and cautious administration of crystalline insulin in small amounts, hypoglycemic episodes were frequent. Blood sugar levels measured at these times varied from 40 mg. per cent to 65 mg. per cent. On her fourth hospital day she was spontaneously delivered of a stillborn infant. Blood loss during delivery was measured at 250 cc. Immediately following this

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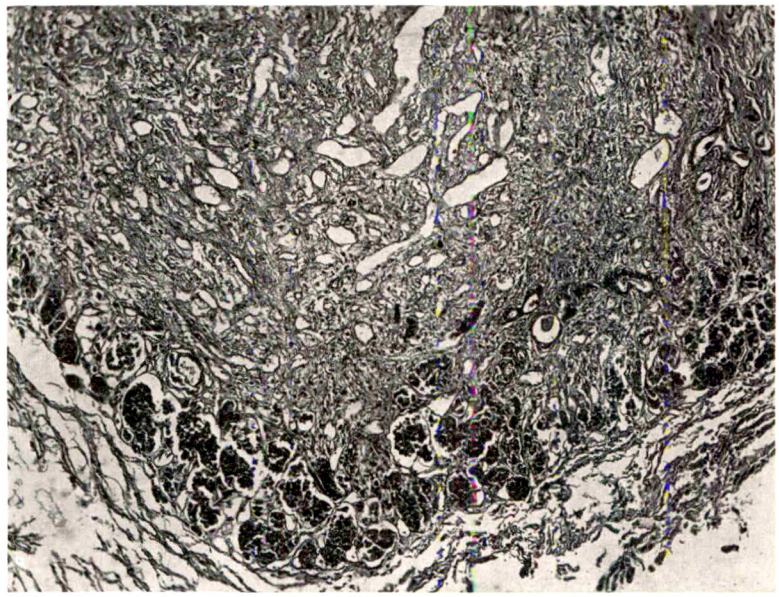


FIG. 1. Section of the pituitary showing extreme destruction and scarring; × 66.

severe hypoglycemic shock occurred which was relieved by intravenous glucose administration. The patient had a sharp rise in temperature to 105°F. From this time until her death two and a half weeks later there was intermittent fever. Hypoglycemic episodes occurred usually at night and blood sugar levels were always found to be between 37 and 65 mg. per cent, although insulin administration had been stopped before delivery. These episodes were accompanied with left-sided fits; they appeared to be made worse by prolonged fast. The patient was weak, listless and anorectic. Glucose was administered intravenously in the last days of life as well as aqueous adrenal-cortical extract. The patient fell into a deep coma six days before death. She never regained consciousness despite glucose administration. It was thought irreversible changes had occurred in the brain as a result of the episodes of profound hypoglycemia. She died quietly three weeks after admission. A diagnosis* of infarction of the pituitary was made and it was suggested that the amelioration of the diabetes mellitus and the subsequent hypoglycemia were secondary to this.

At autopsy she was found to have in addition to low grade endometritis and cystitis, atrophy of the pancreas, hyalinization of the islets of Langerhans, extreme destruction and scarring of the hypophysis secondary to infarction, (Fig. 1) and atrophy of the adrenal cortex. The infarction of the pituitary was estimated to be of two months' duration.

CASE II. G. R. (J. H. H. No. 272744), a sixty-eight year old retired white carpenter, entered the Urological Service of the hospital on October 25, 1952, for a perineal prostatectomy for benign prostatic hypertrophy. There was a strong familial history of diabetes mellitus. He had been found to be a diabetic at the age of fifty-eight years and had been followed in the Diabetic Clinic of the hospital for four years. Regulation was accomplished by attempted dietary control and administration of insulin. In the past he had had one severe bout of acidosis apparently precipitated by severe infection. Before operation, while he was in the hospital receiving a 1,600 calorie diet and 20 units of protamine zinc insulin every morning, several blood sugar determinations, made when he was fasting, were between 140 and 240 mg. per cent. Glycosuria was always present, but without acetonuria. Perineal prostatectomy was performed successfully. On the second postoperative day he had several shaking chills and became febrile. The patient was given penicillin and streptomycin. Fever disappeared within two days. Crepitation and foul-smelling drainage appeared at the operative site on the fifth postoperative day. The diabetes became difficult to control. Blood sugar level rose to between 220 and 320 mg. per cent. He became mildly

*Made by Dr. James Bordley, III, then Resident Physician.

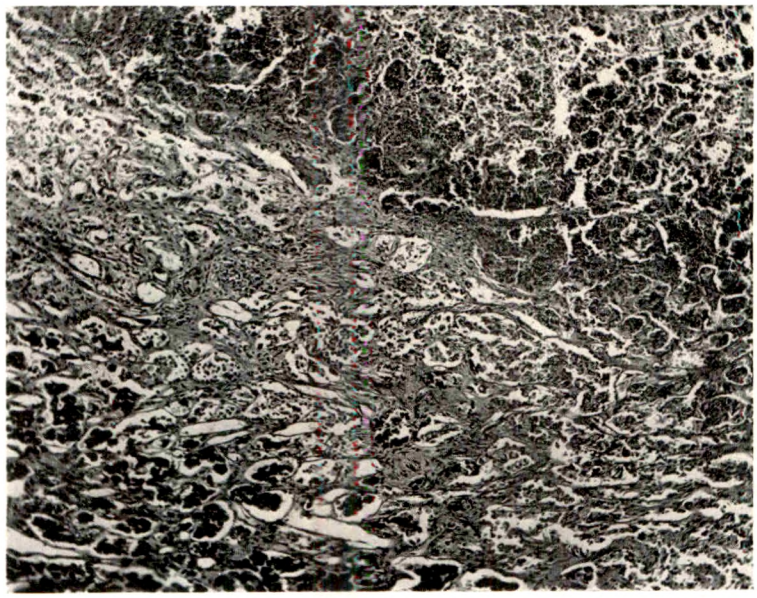


FIG. 2. Section of the pituitary showing abscess formation and necrosis in the anterior lobe; × 66.

acidotic. Insulin dosage was increased from 20 to 55 units of protamine zinc insulin every morning, but glycosuria persisted and blood sugar levels remained high. Streptococcus faecalis and a group D beta hemolytic streptococcus were cultured from the perineal drainage. With antibiotic therapy the fever and tachycardia disappeared and the fasting blood sugar levels returned to normal on the eleventh postoperative day. Insulin dosage was decreased from 55 to 10 units of protamine zinc insulin every morning. Blood sugar levels fell below normal and insulin administration was discontinued on the fourteenth postoperative day; glycosuria never reappeared. Low grade fever returned. On the sixteenth postoperative day the patient complained of mild headache, ocular pain and photophobia. This increased in severity for the next four days. Fever, tachycardia and tachypnea reappeared. The patient became weak and listless; anorexia increased and vomiting occurred. Examination of the eye grounds showed slight pallor of the discs and slight fullness of the retinal veins. On the twentieth postoperative day the patient became comatose. A varying neurologic pattern appeared with generalized flaccidity, twitching of the facial and pectoral muscles, absent deep tendon reflexes and unsustained bilateral ankle clonus. It was thought this picture represented extreme hypoglycemia and, after blood was obtained for a sugar determination, 100 gm. of glucose was administered intravenously in a 10 per cent solution, but the patient died four hours later. The blood sugar concentration was subsequently found to be 32 mg. per cent.

At autopsy in addition to generalized arteriosclerosis, atrophy of the pancreas and hyalinization of the islands of Langerhans, there was abscess formation at the operative site in the perineum and abscess and necrosis of the anterior lobe of the hypophysis. (Fig. 2.) Str. faecalis was cultured from the abscesses in the pituitary and in the perineum.

CASE III. A. M. (J. H. H. No. 533662), a forty year old Negro laborer, was last in the hospital in April, 1953. There was a strong family history of diabetes mellitus but of no other endocrine disorders. The patient had syphilis at the age of fifteen years. This was treated with penicillin during his first hospital admission in 1950.

In 1949 he was admitted to another hospital in severe diabetic acidosis. He had had symptoms of diabetes for the preceding six months. The diabetes was well controlled by dietary regulation and insulin administration, 50 units of protamine zinc insulin and 30 units of crystalline insulin every day. During that hospital stay he had an excruciating headache lasting for several days. There were no abnormal physical findings associated with this. Following discharge he took insulin sporadically. He first appeared at

The Johns Hopkins Hospital in 1950. At the Wilmer Ophthalmological Institute he was found to have interstitial keratitis, iritis, lymphadenopathy and hepatomegaly. There were no visual field defects. Biopsy of an axillary gland showed lesions compatible with the diagnosis of sarcoid. The blood sugar determinations obtained when the patient was fasting were all normal and the oral glucose tolerance test was normal. He had stopped taking insulin and had given up dieting some six months previously. At no time was there glycosuria. Several determinations of the basal metabolic rate yielded results of approximately −30 per cent of normal. The serum concentrations of sodium, potassium and chloride were always normal. There was mild normochromic-normocytic anemia, and a hypoplastic bone marrow. X-ray of the sella turcica showed it to be intact and at the upper limits of normal in size.

The patient left the hospital but returned again in February, 1953, complaining of weakness, dizziness, crampy abdominal pain and watery diarrhea. Anorexia was prominent although there was no nausea or vomiting. On physical examination he was found to have low grade fever and mild hypotension. Blood pressure was 90/60 mm. Hg, recumbent. He appeared lethargic and chronically ill. The skin was dry and scaly. No increase in pigmentation was noted. Facial, axillary and pubic hair was sparse. There was generalized lymphadenopathy. The liver was palpable at the costal margin. The testes were moderately atrophied. Determinations of blood sugar levels made when the patient was fasting were always normal and the oral glucose tolerance curve was flat, but the intravenous glucose tolerance curve was diabetic in type. The severe normochromic-normocytic anemia discovered during his first admission in 1950 was again noted. The protein-bound iodine was 2.4 μg./100 cc. of serum.* 17-Ketosteroid levels in the urine were 2.2 mg./24 hours and 1.0 mg./24 hours on two occasions.† Serum levels of sodium, chloride and potassium were normal. X-rays of the skull showed a normal sella. Visual fields were normal. Blood transfusions corrected the anemia. It was recommended that the patient take replacement hormonal therapy (thyroid, cortisone and testosterone) but this advice was refused. The patient continues with signs of increasing pituitary insufficiency manifested by weakness, lassitude, slow mentation, loss of hair, loss of libido, atrophy of genital organs, coarse skin, intolerance to cold, and hypotension.

He has generalized sarcoidosis and it is believed that there is involvement of the anterior lobe of the pituitary by this process. Clinically he presents a picture of pituitary insufficiency of moderate degree. The amelioration of the diabetes is thought to be the result of the pituitary insufficiency.

* Normal value: 4.0 to 8.0 μg./100 cc. of serum.

† Normal value for males of this age: 8.0 mg./24 hours to 14.0 mg./24 hours.

DISCUSSION

In 1930 Houssay and Biasotti reported that the diabetes produced by extirpation of the pancreas in toads could temporarily be prevented or its severity diminished by removal of the pituitary gland.[2] In 1931 they reported that in dogs diabetes mellitus produced by extirpation of the pancreas could be ameliorated by removal of the anterior lobe of the pituitary; in addition they found that such dogs were hypersensitive to insulin and that brief fasting caused severe hypoglycemia.[3] Other investigators have confirmed these findings in other mammals, amphibians, reptiles and fishes.[4] The glucose tolerance curves in such animal preparations may remain diabetic in type.[5] In 1937 Young reported that large amounts of pituitary extract given to dogs over a prolonged period of time produced permanent diabetes.[6] The pancreases of these diabetic dogs showed either degranulation and hyalinization of the beta cells alone or hyalinization of all islet tissue.[7] Gaebler and Galbraith found that in dogs rendered diabetic by pancreatectomy, but well controlled by insulin administration, anterior pituitary extract caused worsening of the diabetic state and made control difficult.[8]

In 1940 Gemmill showed that rat diaphragm incubated in a glucose-containing medium led to more substantial glycogen deposition in the diaphragm when insulin was present than when insulin was absent.[9] Using this technic Stadie and his co-workers found that the ease with which insulin combines with the tissue is impaired in the diaphragm of rats injected with crude anterior pituitary extract or purified growth hormone before sacrifice.[10] Nelson independently has made the same observation.[11] Price, Cori and Colowick reported that the addition of anterior pituitary extract to extracts

of rat tissues produced inhibition of the hexokinase activity of these tissues, and that when insulin was added to this reaction, the inhibition was counteracted.[12] Whether the action of anterior pituitary extract is through inhibition of hexokinase activity is not certain for Stadie and Haugaard[13] could not confirm this experiment of Price et al.

Whatever the exact mechanism, it is abundantly clear that there is a mutual antagonism between the effects of insulin and an anterior pituitary hormone upon the concentration of blood glucose in the animal body. It is not surprising then to find this same antagonism in man,[14,15] with amelioration of diabetes in diabetic individuals who develop anterior pituitary insufficiency. Although Calder[16] is usually given credit for reporting the first instance in man in 1932, Balo[17] had reported two such cases in 1924. Since then eleven cases of amelioration of diabetes associated with spontaneous destruction of the anterior pituitary gland have been reported in the literature,[18–28] also two cases of amelioration of diabetes after the anterior pituitary gland had been removed surgically.[29,30] These reports are summarized in Tables I and II.

Spontaneous pituitary insufficiency and amelioration of diabetes has shown no sex predilection. It has occurred in both juvenile and senile patients with diabetes mellitus. Of the reported cases five were males and six were females. Five individuals were under thirty. In the cases reported in this communication two were males, aged forty and sixty-eight years, respectively, and one was a female aged twenty-two years.

The duration of diabetes before the development of the pituitary insufficiency in the previously reported cases varied from four months to twenty-one years. The majority had had diabetes for at least four years. In the three cases reported here the diabetes had been present five, nine and ten years before any signs of the pituitary lesion developed.

Among the six females reported in the literature, the pituitary insufficiency occurred concomitantly with pregnancy or with postpartum hemorrhage in four. In the one female reported in this communication the pituitary lesion developed during pregnancy.

The complaint common to all of the patients reported here and in several of the previously reported cases was severe headache. In retrospect this complaint was made at the time infarction must have occurred in the pituitary. Attacks of hypoglycemia quickly followed and were usually not initially recognized as attacks of hypoglycemia. They were ushered in by delirium, fits or coma. Rapidly changing neurologic signs conforming to no set pattern appeared in all cases. Because of these prominent neurologic signs attention was usually focused on the central nervous system. Generally, a low level of the blood sugar was a surprising incidental finding. When hypoglycemia was discovered its full significance was usually unappreciated. Despite reduction in insulin dosage, hypoglycemia attacks usually continued. Within seven to ten days most patients had discontinued insulin completely. Despite this, glycosuria did not appear except when large amounts of glucose were given intravenously. In all patients the manifestations of hypoglycemia were profound and the symptoms were quite refractory to treatment with intravenously administered glucose. Moreover, hypoglycemia quickly recurred when the intravenous administration of glucose was stopped.

Of the eleven previously reported cases nine of the patients have died and have come to autopsy. In eight massive infarction of the pituitary gland secondary to thrombosis of its nutrient arteries was found. Death occurred in these eight patients as early as a week or as late as six months after hypoglycemic attacks began. In the ninth case (Case 3, Table I) death occurred twelve years after the onset of hypoglycemic attacks. When the first hypoglycemic attacks occurred, the patient was thought to have a pituitary tumor. X-rays of the skull then showed "definite evidence of a pituitary tumor—an abnormally dense deposit lying in the sella—and a lateral stereo confirmed the position of the radio-opaque mass." Insulin was no longer required. Three years later glycosuria and hyperglycemia recurred and administration of insulin was again required in increasing dosages. The patient lived nine years longer, requiring up to 30 units of crystalline insulin every day. He finally died in diabetic coma. At autopsy there was no sign grossly of pituitary enlargement or tumor and microscopically the gland was normal.[19]

Of the three cases reported in this communication two have come to autopsy. The female had anterior pituitary infarction; she lived only six weeks after attacks began. The male had an abscess destroying the pituitary gland, metastatic

TABLE I

AMELIORATION OF DIABETES MELLITUS FOLLOWING SPONTANEOUS DEVELOPMENT OF PITUITARY INSUFFICIENCY
(SUMMARY OF PREVIOUSLY REPORTED CASES)

Case No., Author and Year Reported	Age and Sex	Pregnancy	Duration Diabetes (Yr.)	Duration Development Pituitary Insufficiency	Headache Presenting Complaint	Signs of Pituitary Insufficiency								Decrease in Insulin Requirement	Glucose Tolerance Curves after Pituitary Insufficiency	Antemortem Diagnosis	Hormonal Replacement Therapy	Fate	Autopsy Findings			
						Weakness	Lassitude	Lowered Blood Pressure	Hair Loss	Atrophy Genital Organs	Breast Atrophy	Signs of Hypothyroidism	Hypoglycemia						Infarction Anterior Pituitary	Hyalinization Islet Tissue	Adrenal-cortical Atrophy	Other
1. Balo, 1924	16♂	.....	3	2½ mo.	0	..	?	?	..	..	..	..	+	.....	Not done	No	None	Died 2½ mo. after onset hypoglycemic attacks	+	+	+	Pulmonary Tbc
2. Balo, 1924	28♀	No	1½	1 mo.	0	..	?	?	..	..	..	..	+	.....	Not done	No	None	Died 1 mo. after onset of hypoglycemic attacks	+	+	+	Pulmonary Tbc
3. Lyall and Innes, 1933	27♂	.....	½	2 mo.	0	+	+	+	+	+	..	0	+	30 U ↓ 0 U	Remained diabetic	Yes	Anterior pituitary extract infrequently	Lived 12 yr.; 3 yr. after pituitary insufficiency appeared, diabetes reappeared; patient died in diabetic acidosis	No (normal pituitary)	+	No	.....
4. Kotte and Vanderache, 1940	40♂	.....	5	2 wk.	+	+	+	+	0	0	..	0	+	? ↓ 0 U	Not done	No	None	Died suddenly in profound hypoglycemia 12 hr. after hospital admission	+	+	+	Pulmonary Tbc
5. Feldman, Susselman, Roberts and Lipitz, 1947	50♀	No	12	wk.	+	+	+	+	+	+	+	0	+	No insulin taken at any time	Not done	Yes (Simmonds' disease)	Adrenocortical extract daily	Died 4 mo. after onset of symptoms in profound hypoglycemia	+	+	+	.....
6. Vanderbroech and Ferin, 1947	35♀	Yes	5	6 mo.	+	+	+	+	+	+	+	+	+	60 U ↓ 40 U	.....	Yes (Sheehans' disease)	Gonadotrophins weekly	Alive but ill; replacement therapy not satisfactory	.....	..	.....	.....
7. Marzullo and Handlesman, 1951	53♂	.....	1½	2 wk.	+	+	+	+	0	+	+	..	+	165 U ↓ 20 U	Remained diabetic	No (? Addison's disease)	Desoxycorticosterone acetate daily to death	Died 6 wk. after onset of hypoglycemic attacks	+	+	+	.....
8. Williams, 1952	27♀	Yes	4	16 days	0	+	+	+	0	?	0	0	+	60 U ↓ 0 U	Not done	No	None	Died 16 days after hypoglycemic attacks began in profound hypoglycemia resistant to I.V. glucose therapy	+	+	+	.....

TABLE I (*Continued*)

Case No., Author and Year Reported	Age and Sex	Pregnancy	Duration Diabetes (Yr.)	Duration Development Pituitary Insufficiency	Headache Presenting Complaint	Signs of Pituitary Insufficiency								Decrease in Insulin Requirement	Glucose Tolerance Curves after Pituitary Insufficiency	Antemortem Diagnosis	Hormonal Replacement Therapy	Fate	Autopsy Findings			
						Weakness	Lassitude	Lowered Blood Pressure	Hair Loss	Atrophy Genital Organs	Breast Atrophy	Signs of Hypothyroidism	Hypoglycemia						Infarction Anterior Pituitary	Hyalinization Islet Tissue	Adrenal-cortical Atrophy	Other
9. Israel and Conston, 1952	30 ♀	Yes	16	6 mo.	0	+	+	+	+	0	0	+	+	20 U ↓ 0 U	Not done	No	None	Died suddenly following cesarean section; pituitary insufficiency developed after a postpartum hemorrhage 3 yr. previously	+	+	Slight	.....
10. Poulson, 1953	38 ♀	Postpartum	21	6 mo.	0	+	+	+	+	+	+	+	+	30 U ↓ 8 U	Remained diabetic	Yes (Sheehan's disease)	Cortisone, insulin, thyroid extract } daily Percorten, stilbesterol } weekly	Alive and well 9 yr. after presumed postpartum necrosis of anterior pituitary; replacement therapy satisfactory	.....	..	.....	.....
11. Rothfeld and Rodas, 1954	65 ♂	.....	9	2 yr.	0	+	+	+	+	+	+	+	+	25 U ↓ 0 U	Remained diabetic	No	None	Insufficiency developed 2 yr. prior to admission; last 9 mo. of life symptoms became worse; rapid progression to death	+	+	+	.....

TABLE II

AMELIORATION OF DIABETES MELLITUS BY SURGICAL REMOVAL OF THE ANTERIOR PITUITARY GLAND

Case, Author and Year Reported	Age and Sex	Duration Diabetes before Hypophysectomy (yr.)	Date Hyphophysectomy	Results of Hypophysectomy				Hormonal Replacement Therapy	Fate	Autopsy
				Decrease Insulin Requirement	Hypoglycemic Episodes	Glucose Tolerance Test	Other Signs of Pituitary Insufficiency			
1. Chabanier Puech, Lobo-Onell, Lelu, 1935	22 ♂	7	Oct., 1934	Yes 170 U ↓ 70 U	Frequent	Not done	Yes	None	Died 4 mo. after operation in hypoglycemia	Absent pituitary; pulmonary tuberculosis
2. Luft and Olivecrona, 1953	30 ♂	28	Nov., 1951	Yes 80 U ↓ 12 U	None	Not done	Yes	Cortisone, doca, thyroid, testosterone	Alive and well 2 yr. after operation	
3. Luft and Olivecrona, 1953	20 ♀	14	May, 1952	Died postoperatively						
4. Luft and Olivecrona, 1953	28 ♀	17	May, 1952	Died postoperatively						
5. Luft and Olivecrona, 1953	24 ♀	14	June, 1952	Yes 60 U ↓ 8 U	Yes	Not done	Yes	Cortisone, doca, thyroid	Died (suddenly) 1 mo. after operation	Not done

from a perineal abscess with septicemia; he died within ten days of the onset of symptoms.

Among the cases reported in the literature, two persons (Cases 6 and 10, Table 1) are still alive.[23,27] Each is presumed to have an infarction of the anterior pituitary. Both suffered postpartum hemorrhage. Each quickly developed many signs of pituitary insufficiency including weakness, lassitude, anorexia, vomiting, hypotension, loss of hair, loss of libido, atrophy of clitoris and labia, amenorrhea, atrophy of breasts, thickened skin, intolerance to cold and mental slowness. These individuals have survived nine and seven years, respectively, with hormonal replacement therapy which has included percortin, thyroid, stilbestrol and, more recently, cortisone. The only surviving patient among our three cases has clear-cut signs of thyroid, adrenal and gonadal hormone deficiencies. He has generalized sarcoidosis and is presumed to have involvement of the anterior pituitary by this process, a not unknown occurrence.[31] He has survived now for three years without replacement therapy.

In five of the previously reported cases and in the three cases reported here the administration of insulin was discontinued within ten days of onset of symptoms. In three other cases previously reported the dosage of insulin was greatly reduced. One patient (Case 5, Table 1) never required insulin and Balo's cases (Cases 1 and 2, Table 1) occurred before insulin was available.

Despite the fall in requirement for insulin some abnormality in the metabolism of carbohydrate persists. In only two of the individuals who no longer required insulin were glucose tolerance tests performed. The curves remained diabetic in type. The intravenous glucose tolerance test in our patient with sarcoid remained diabetic in type. The flat curve after sugar was administered orally indicates to us delayed intestinal absorption.

There are two reports in the literature of surgical removal of the anterior pituitary gland to ameliorate the complications of diabetes mellitus.[29,30] Only two of five individuals have survived the operation. In both of these individuals insulin requirement was somewhat reduced. One individual died four months after the operation, in hypoglycemia. He had pulmonary tuberculosis in addition to diabetes. At autopsy he had slight adrenal-cortical atrophy but no tuberculous involvement of the adrenal glands. The other patient is alive two years after operation. His insulin requirement is 12 units of protamine insulin daily instead of 80 units of protamine insulin. His diabetic state is reported as less erratic and more stable. He requires replacement therapy consisting of cortisone, desoxycorticosterone acetate, desiccated thyroid and testosterone.

SUMMARY

The Houssay phenomenon, so clearly described in experimental animals, has been seen but rarely in man. A study of this phenomenon in man has been made from the review and follow-up of the eleven cases previously reported in the literature and from recent observations upon three individuals who exhibited this phenomenon. This condition, when it occurs, usually involves individuals who have had diabetes mellitus for a considerable time. It is caused usually by infarction of the anterior lobe of the pituitary gland. One of the first effects of the anterior pituitary insufficiency is hypoglycemia. It follows within twenty-four hours after the infarction occurs. In these individuals the hypoglycemia is manifested by bizarre and rapidly changing neurologic signs associated with unconsciousness. These individuals exhibit extreme sensitivity to administered insulin. Those surviving the acute phases of this illness began within a month or two to exhibit the clinical manifestations of deficiencies of thyroid, adrenal and gonadal hormones. Diabetes in these individuals is by no means cured but only ameliorated for the diabetic tendency remains, although the symptoms of diabetes disappear, glycosuria decreases and the level of sugar in the blood approaches the normal level. The response to the stress of intravenously administered glucose is still diabetic in type. Continued administration of insulin to these individuals is sometimes required but the "Houssay man" is quite as sensitive to insulin as the "Houssay dog," and hypoglycemia is always a threat to life.

Acknowledgment: We wish to thank Dr. James Bordley, III, for his permission to publish his case.

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Biochemical Alterations during Steroid Hormone Therapy of Advanced Breast Cancer*

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STEROID hormones and castration are successfully employed in the palliative management of advanced carcinoma of the breast.[1–4] Objective improvement occurs in that primary and metastatic lesions decrease in size or disappear, and distressing subjective symptoms diminish in intensity.

In conjunction with an investigation of the clinical and pathologic alterations in advanced breast carcinoma associated with steroid hormone therapy, the accompanying metabolic and biochemical changes were investigated. It was apparent that such studies would aid in the interpretation of the mechanisms of action of the agents employed, as well as serve as a guide to therapy. This report comprises a record and interpretation of the biochemical alterations observed during hormone therapy of advanced breast cancer, and an evaluation of their use in the management of these patients.

Of the patients investigated, a small number were studied intensively on a metabolic ward;[4–8] the majority were studied in the Tumor Clinic. Large doses of androgenic or estrogenic hormones were administered following preliminary observations. No vitamins, iron or other treatment was given, nor were more definitive methods of therapy, such as irradiation, employed during the period of study. Blood chemical determinations were performed at intervals of three days to four weeks, the analytic methods being those employed in the chemical laboratories of the Massachusetts General Hospital.[9]

CALCIUM

Initial Serum Calcium. Initial or pretreatment serum calcium determinations were obtained in 111 patients. Hypercalcemia was considered to be present when the serum calcium exceeded 11.0 mg. per cent, as was the case in eight patients (7.2 per cent). (Table I.) Of the 111 patients, osseous metastases were demonstrated in fifty patients, of whom six (12 per cent) had hypercalcemia. In two patients with elevated serum calcium, osseous metastases were not detected on roentgen examination. In one of these patients the serum calcium initially was 15.9 mg. per cent and skeletal pain was present; the serum calcium decreased and the pain disappeared on institution of androgenic hormone therapy. Both patients died soon after the onset of hormone therapy from progression of the disease. Postmortem examination revealed extensive osseous metastases in both instances. In no patient with hypercalcemia was there elevation of the serum protein. Marked hypocalcemia was not encountered in this study.

TABLE I
INITIAL (PRETREATMENT) SERUM CALCIUM VALUES*

Serum Calcium (mg. %)	Osseous Lesions	Soft Tissue Lesions Only	Total Patients
Below 8.0	0	1	1
8.0–9.0	8	9	17
9.0–10.5	35	49	84
10.5–11.0	1	0	1
Over 11.0	6	2	8
Total	50	61	111

* Normal serum calcium: 8.5 to 10.5 mg. per cent. An initial "spontaneous" hypercalcemia was present in 12.0 per cent of the patients with known osseous metastases.

* From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University and the Tumor Clinic at the Massachusetts General Hospital, Boston, Mass. This is publication No. 845 of the Cancer Commission of Harvard University. This work was supported in part by an institutional grant to the Massachusetts General Hospital from the American Cancer Society and a grant from the National Cancer Institute of the U. S. Public Health Service.

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Serum Calcium during Hormone Therapy. Alterations of the serum calcium were observed in ninety-three patients over a period of several months during estrogenic or androgenic hormone therapy. (Table II.) A decrease of more than 1.0 mg. per cent in the serum calcium was noted in fifteen patients with and without demonstrable osseous metastases. No significant hypocalcemia was encountered. All the patients who exhibited pretreatment hypercalcemia demonstrated a marked decrease of serum calcium to normal levels. There was no accentuation of existing hypercalcemia.

TABLE II
SERUM CALCIUM ALTERATIONS OF MORE THAN 1.0 MG. PER CENT DURING STEROID HORMONE THERAPY

Hormone Employed	Serum Calcium	Osseous Lesions (x-ray)	Soft Tissue Lesions Only	Total Patients
Androgen	Decreased	4	2	6
	No change	16	7	23
	Increased	6	1	7
Estrogen	Decreased	2	7	9
	No change	13	32	45
	Increased	2	1*	3
Total	Decreased	6	9	15
	No change	29	39	68
	Increased	8	2	10
			Total	93

* Remained within normal range.

Ten of the ninety-three patients had an increase of serum calcium of more than 1.0 mg. per cent during hormone therapy. Eight of these had demonstrable osseous metastases. Seven patients developed serum calcium levels over 11.0 mg. per cent and demonstrated the characteristic syndrome of "induced hypercalcemia."[6] Clinically this consisted of apathy, anorexia, nausea, vomiting and drowsiness. Weight loss, dehydration, disorientation, stupor. coma, vascular collapse and death may occur with severe hypercalcemia. Laboratory studies of the "induced hypercalcemia" state revealed an increase in serum and urinary calcium and a negative calcium balance. No elevation of serum protein accompanied this change.

There was no significant alteration of serum calcium in sixty-eight patients during hormone therapy.

PHOSPHORUS

Initial Serum Phosphorus. The serum phosphorus was recorded in 113 patients before hormone therapy was instituted. (Table III.) Only fourteen had a phosphorus level over 4.5 mg. per cent. Two of these patients had an associated hypercalcemia. However, the two patients with values over 6.0 mg. per cent had normal serum calcium levels though osseous metastases were present. In patients with an elevated serum phosphorus, the serum nonprotein nitrogen was normal. Hypophospha-

TABLE III
INITIAL SERUM PHOSPHORUS VALUES (PRETREATMENT)*

Serum Phosphorus (mg. %)	Osseous Lesions	Soft Tissue Lesions Only	Total Patients
Below 3.0	7	16	23
3.0–4.5	35	41	76
4.5–6.0	8	4	12
Over 6.0	2	0	2
Total	52	61	113

* Normal serum phosphorus, 3.0 to 4.5 mg. per cent. The two patients with values over 6.0 mg. per cent had normal serum calcium and NPN results. The highest value was 6.8 mg. per cent, the lowest 2.2 mg. per cent.

TABLE IV
PHOSPHORUS ALTERATIONS OF MORE THAN 1.0 MG. PER CENT DURING STEROID HORMONE THERAPY*

Hormone Employed	Serum Phosphorus	Osseous Lesions	Soft Tissue Lesions	Total Patients
Androgen	Decreased	15	2	17
	No change	5	2	7
	Increased	3	0	3
Estrogen	Decreased	10	27	37
	No change	7	15	22
	Increased	0	0	0
Total	Decreased	25	29	54
	No change	12	17	29
	Increased	3	0	3
			Total	86

* A decrease occurred in 62.8 per cent.

temia of less than 3.0 mg. per cent was noted in twenty-three patients, the lowest level being 2.2 mg. per cent.

Serum Phosphorus during Hormone Therapy. An initial increase in the serum phosphorus of more than 1.0 mg. per cent occurred in three of eighty-six patients during steroid hormone therapy. (Table IV.) These patients were receiving testosterone. There was no evidence of renal disease or hypercalcemia, although each of the patients in question had osseous metastases. No increase in serum phosphorus was noted during the early period of estrogenic therapy.

A decrease in serum phosphorus of more than 1.0 mg. per cent occurred in fifty-four (62.8 per cent) patients during the administration of hormones. (Table IV.) Of these patients, twenty-seven receiving estrogenic hormone and two receiving androgenic hormone had no demonstrable osseous metastases. In one patient exhibiting the "induced hypercalcemic syndrome" there was an increase in urinary phosphorus, decrease of serum phosphorus and an increase in serum and urinary calcium.

ALKALINE PHOSPHATASE

Initial Serum Alkaline Phosphatase. Serum alkaline phosphatase determinations were carried out in 110 patients before hormone therapy was instituted. Of the forty-seven patients in whom osseous metastases were known to be present, twenty-eight (59.6 per cent) had alkaline phosphatase values over 4.5 Bodansky units (B.U.), and nineteen (40.4 per cent) had values over 6.0 B.U. One patient in the latter group had extensive liver metastases which probably accounted for the elevated phosphatase. (Table V.)

There were sixty-three patients in whom no osseous metastases could be detected on roentgen examination and who had no clinical manifestations of bone involvement. The alkaline phosphatase was elevated above 4.5 B.U. in thirty-seven (57.4 per cent) of these patients, and twenty-two (34.9 per cent) had values over 6.0 B.U. Of this latter group, six patients manifested osseous metastases by x-ray or clinical signs within a few weeks, five had enlarged livers due to metastases, and two later manifested hepatic metastases by enlargement of the liver. Two patients with primary tumors of the breast had extensive Paget's disease of the bone associated with unusual high alkaline phosphatase values. In the remaining seven patients no explanation for the elevated alkaline phosphatase was found.

Serum Alkaline Phosphatase during Hormone Therapy. The alkaline phosphatase was determined serially in ninety-eight patients during hormone therapy. (Table VI.) A decrease in phosphatase level of more than 2 B.U. occurred in twelve patients. There was no apparent correlation with the presence or absence of osseous metastases or the hormones employed. One twenty-seven year old woman with liver metastases, however, demonstrated a decrease in size of an enlarged liver and a decrease in serum alkaline phosphatase during androgen therapy.

An increase in serum alkaline phosphatase of more than 2 B.U. during hormone therapy occurred in twenty-four (53.3 per cent) of the forty-five patients with demonstrable osseous metastases. In only one of these was this due to the presence of obvious hepatic metastases. The increase in alkaline phosphatase activity usually occurred during the first three months of therapy, and after four months tended to decrease, sometimes to pretreatment levels. In one such patient the unusually high value of 71.2 B.U. was recorded shortly after the onset of therapy. The increase in serum alkaline phosphatase was associated with evidence of apparent healing of the osseous lesions, consisting of calcification of previously osteolytic lesions or a more normal appearing osseous structure. Excluding patients with hepatic involvement, of those patients with osseous metastases who had

TABLE V
INITIAL (PRETREATMENT) ALKALINE PHOSPHATASE VALUES*

Alkaline Phosphatase (B.U.)	Osseous Metastases	Soft Tissue Metastases	Total Patients
Normal	19	26	45
4.5–6.0	9	15	24
Over 6.0	19†	22‡	41
Total	47	63	110

* Normal serum alkaline phosphatase 2.0–4.5 Bodansky units per cent.

† One of these had associated liver metastases which partially accounted for the increase.

‡ Of these, six patients manifested osseous metastases within a few weeks, five had enlarged livers due to metastases, two later manifested hepatic metastases, and two had extensive Paget's disease of the bones.

an elevation in serum alkaline phosphatase, 65 per cent (fifteen patients) demonstrated evidence of bone repair. (Table VII.) This is 33 per cent of the forty-five patients treated, a slightly higher incidence of bone repair than the 23 per cent recorded in a larger series.[2]

TABLE VI
ALTERATIONS OF MORE THAN 2 B.U. IN SERUM ALKALINE PHOSPHATASE DURING STEROID HORMONE THERAPY

Hormone Employed	Alteration of Alkaline Phosphatase Greater than ±2 B.U.	Osseous Metastases	Soft Tissue Metastases	Total Patients
Androgen	Decreased	3*	1	4
	No change	7	6	13
	Increased	14†	1†	15
Estrogen	Decreased	3	5	8
	No change	8	31	39
	Increased	10	9‡	19
Total of both hormones	Decreased	6	6	12
	No change	15	37	52
	Increased	24	10	34
	Total	45	53	98

* One patient of this group had liver metastases that decreased in size during testosterone therapy.

† One patient in each of these groups developed liver metastases during hormone therapy that continued to increase in size.

‡ In five patients liver metastases continued to increase in size during therapy to account for this increase, and in 4 patients osseous metastases became manifest during hormone therapy.

An elevation of the serum alkaline phosphatase during hormone therapy occurred in ten of fifty-three patients in whom no osseous metastases were demonstrated. Of these patients, six had progressive enlargement of the liver due to metastases. In the remaining four patients osseous metastases of an osteoblastic type were recognized by x-ray during the course of hormone therapy. Presumably osseous metastases were present in these four patients prior to the institution of hormone therapy. As the result of regression of the tumor and recalcification of the area, dense areas became visible in x-ray films.

ACID PHOSPHATASE

Elevation of the serum acid phosphatase in metastatic carcinoma of the prostate has been shown to be of diagnostic and clinical value. With tumors other than prostatic carcinoma, an increase of serum acid phosphatase activity is occasionally encountered, most frequently in carcinoma of the female breast with osseous metastases.[10] Such an increase was noted in the present study in patients with osseous metastases.

TABLE VII
CORRELATION OF THE INCREASE IN SERUM ALKALINE PHOSPHATASE WITH RESPONSE OF OSSEOUS LESIONS DURING HORMONE THERAPY OF ADVANCED BREAST CANCER WITH OSSEOUS METASTASES

Hormone Employed	Progression of Disease	No Change in Disease	Improvement	
			Moderate	Excellent
Testosterone (13 patients)	4	....	4	5
	30.7%	....	30.7%	38.4%
			69.1%	
Estrogen (10 patients)	3	1	2	4
	30%	10%	20%	40%
			60%	

Acid phosphatase values were determined in the sera of forty-three women with carcinoma of the breast, twenty-seven of whom showed osseous metastases by x-ray examination. Twenty of this latter group had acid phosphatase values over 3.0 Gutman units per cent, while those without osseous lesions showed normal values. The sera of thirty other women gave values below 3.0 units per cent, except those with parathyroid adenomas or Paget's disease.

Two patients demonstrated an unusual alteration of serum acid phosphatase during the administration of testosterone propionate. Patient J. W. was a sixty-five year old woman with extensive osseous metastases studied on the metabolic ward. Initial studies revealed: serum calcium 8.9 mg. per cent, phosphorous 3.9 mg. per cent, alkaline phosphatase 10.2 Bodansky units and acid phosphatase 7.8 Gutman units per cent (normal values 1 to 3 units per cent). Testosterone propionate, 100 mg. intramuscularly, was administered three times a week. At the end of one month the serum alkaline phosphatase was 5.8 B.U. and the acid phosphatase 13.0 Gutman units. At the end of the second month the serum alkaline phosphatase was

5.9 B.U. and the acid phosphatase 9.3 Gutman units. Throughout this period there was continued progression of the tumor as manifested by increased bone destruction. There was no alteration of the serum calcium or phosphorus. Near the end of the third month of therapy an "induced" hypercalcemia developed. The serum calcium was 16.0 mg. per cent, serum phosphorus 3.2 mg. per cent, alkaline phosphatase 8.9 B.U. and acid phosphatase 5.7 Gutman units per cent. A second patient had an increase from 5.0 units of acid phosphatase activity to 7.6 units during testosterone therapy. This also was associated with progression of the disease and an accompanying "induced" hypercalcemia of 13.0 mg. per cent.

NON-PROTEIN NITROGEN

The serum non-protein nitrogen (NPN) was employed as a gross measure of renal function. Of seventy-two patients studied, the NPN was elevated initially in only two patients (2.7 per cent). In one of these the elevation of 48 mg. per cent was due to dehydration, and became normal following hydration. In the other patient with a value of 54 mg. per cent there was an associated spontaneous hypercalcemia. Following initiation of testosterone propionate therapy in this case the serum calcium and serum NPN became normal.

During hormone therapy an elevation of the NPN was noted in two circumstances: (1) the occurrence of hypercalcemia was accompanied by dehydration and a rise in serum NPN. In some of these patients the hypercalcemia and resultant hypercalciuria caused a deposition of calcium in tissues and renal tubules. In one patient this was followed by permanent renal damage. (2) One patient with extensive abdominal metastases demonstrated a rapid increase of the NPN because of obstruction of both ureters by carcinoma.

LIVER FUNCTION TESTS

A limited number of liver function tests were performed prior to and during the period of hormone therapy. The purpose of these tests was to observe changes that might occur with the unusually high doses of estrogenic and androgenic hormones employed, and to determine whether any of the tests would be of value in guiding hormone therapy. It was not the intent to use these determinations as diagnostic procedures for establishing the presence of liver metastases. Since the patients studied had widespread metastatic carcinoma, it is to be presumed that many had hepatic metastases which may have been minimal in extent. Evaluation of the changes of such lesions during hormone therapy would be difficult. For investigational purposes only those patients with obvious hepatic metastases were studied with respect to liver function changes during hormone therapy.

The liver function tests most frequently employed were the estimation of serum proteins, the cephalin flocculation test and the serum alkaline phosphatase. The van den Bergh test was not routinely employed because it appeared to be a gross test of liver function in patients with metastases to the liver and, if elevated, was associated with the obvious presence of hepatic metastases. Dye excretion tests, such as the bromsulfalein test, were not employed since even in the presence of hepatomegaly due to neoplastic disease they do not distinguish between the presence and absence of liver metastases.[11]

Serum Proteins. Of seventy-eight patients, the initial (pretreatment) serum total protein was normal in sixty-nine or 88.4 per cent of the patients. Values below 6.0 gm. were recorded in five patients. Four of these had ascites or pleural effusion, and one had extensive carcinomatous disease. The lowest value was 5.0 gm. per cent. An elevation of the serum protein was noted in four patients, including one with extensive hepatic metastases. The highest value was 8.6 gm. per cent. None of the patients with an elevated serum protein had an associated hypercalcemia.

During hormone therapy no increase in the serum total protein was observed. No patient in whom the "induced hypercalcemic syndrome" developed had an associated increase of the serum total protein. Only two patients demonstrated a significant decrease in total protein; the lowest value being 4.3 gm. per cent. Both patients had rapid progression of the disease during this period. Patients in a terminal state and not receiving hormonal therapy were not studied.

Cephalin Flocculation Test. In forty-four patients the forty-eight-hour cephalin flocculation test was carried out before hormone therapy was initiated. This test was normal in forty-one patients, although three of these had enlarged livers due to metastases. The cephalin floccula-

tion was 3-plus in three patients, two of whom had a palpable liver due to metastases. The other patient had no other evidence of hepatic disease during the ensuing two years.

The three patients with liver metastases demonstrating a normal cephalin flocculation test, and four others in whom liver metastases developed during hormone therapy, had no increase in flocculation in subsequent cephalin flocculation tests. Patients without liver metastases showed no alteration in the test during hormone therapy.

Van den Bergh Test. In fifteen patients serial serum bilirubin determinations were made. Only one was elevated initially, and this patient had a palpable liver and was clinically jaundiced within a week. Three patients with enlarged livers due to metastases initially had normal van den Bergh tests. Despite hormone therapy the disease progressed and the serum bilirubin became elevated. In eleven patients who did not have apparent liver metastases, there was no alteration of the van den Bergh test during hormonal therapy.

An increase in serum bilirubin occurred in association with hypercalcemia in three patients.[6] Values of 1.8, 2.5 and 2.8 were recorded at the peak of the "induced hypercalcemic syndrome." There was no evidence of hepatic failure.

Alkaline Phosphatase. The alteration of serum alkaline phosphatase that occurrs in association with osseous metastases and during hormonal therapy has already been described. The serum alkaline phosphatase is also an index of liver function in the absence of osseous lesions, and on occasion may be of value despite the presence of osseous metastases.

The serum alkaline phosphatase was elevated initially in five patients with extensive metastases without demonstrable osseous lesions. In only one of these (J. M.) were the cephalin flocculation and van den Bergh tests both abnormal. In all five patients an increase of alkaline phosphatase occurred during hormonal therapy as the disease steadily progressed. (Table VIII.)

In two patients with associated osseous lesions there was excellent objective improvement of the disease during hormone therapy in that the liver decreased in size and osseous lesions were more normal on x-ray examination. In one of these (E. K.) the initial alkaline phosphatase was normal, and the slight rise noted during hormone therapy was regarded as due to the increased osteoblastic activity of bone repair. The other patient (Y. L.) with an initial value of 9.2 B.U. had a decrease in alkaline phosphatase to 5.7 B.U. during hormone therapy. This was postulated as due to a decrease in liver damage,

TABLE VIII

SERUM ALKALINE PHOSPHATASE (B.U.) IN PATIENTS WITH KNOWN LIVER METASTASES PRIOR TO AND DURING HORMONE THERAPY

Patient	Age (yr.)	Osseous Metastases	Pre-treatment	During Treatment
E. K.	29	+	3.4	6.7
Y. L.	27	+	9.2	5.7
H. L.	56	0	6.3	39.6
J. M.	61	0	24.6	42.6
A. P.	53	0	15.8	26.5
M. S.	42	0	21.3	47.0
A. S.	33	0	36.4	48.6

but with a value above normal as a result of osteoblastic activity of bone repair.

HEMATOPOIETIC SYSTEM

The administration of androgenic and estrogenic hormones to patients with advanced breast cancer produced alterations in the hematopoietic system. Of note were those changes observed in the hemoglobin, red blood cell count and hematocrit. (Table IX.) The alterations of the latter two mirrored those of the hemoglobin.

TABLE IX

ALTERATION OF HEMOGLOBIN DURING HORMONE THERAPY OF ADVANCED BREAST CANCER

Hormone Employed	De-crease >2 gm.	No Change ±2 gm.	In-crease >2 gm.	Total Patients
Estrogen				
Number of patients	6	32	4	42
Per cent	14.2	76.2	9.5	..
Androgen				
Number of patients	0	17	16	33
Per cent	....	51.5	48.5	..

Repeated blood examinations of forty-two patients receiving estrogenic hormones showed that in 76.2 per cent (thirty-two patients) the hemoglobin varied less than plus or minus 2 gm.

Six patients (14.2 per cent) had a decrease of more than 2 gm. during therapy and this was associated with progression of the disease. Four patients (9.5 per cent) had an increase of more than 2 gm. in the hemoglobin. Three of these patients had extensive osseous metastases. All four patients had striking clinical improvement as a result of hormone therapy. The highest values recorded were a hemoglobin of 15.4 gm. and 6.8 million red blood cells. In one patient with osseous metastases which improved, the hemoglobin rose from 6.4 to 12.0 gm. In the patients with an increase in hemoglobin and red blood cell count, the bone marrow activity was within normal limits.

Androgenic hormone therapy was associated with an unusual alteration in erythropoietic activity. Of thirty-three patients receiving androgen therapy, seventeen (51.5 per cent) had a decrease of the hemoglobin of not more than 1.0 gm. or an increase of less than 2.0 gm. No patient had a decrease of more than 1.0 gm. despite progression of the tumor. Sixteen patients (48.5 per cent) had an increase of hemoglobin of more than 2.0 gm. This increase was not necessarily accompanied by improvement of the disease nor could it be correlated with the presence or absence of osseous metastases. There was an apparent dissociation between the clinical course of the tumor and the increase in red blood cell activity which appeared. The highest value recorded was a hemoglobin of 18.0 gm., 6.5 million red blood cells and a hematocrit of 59 per cent. The greatest increase in any one patient was from 11.3 to 18.0 gm. of hemoglobin. These changes appeared to be a result of hormone therapy. No transfusions, iron or supplemental vitamins were administered. Bone marrow aspirations revealed hyperplasia of the erythrocyte series.

The increase in erythrocyte activity on administration of large doses of androgen therapy was accompanied by unusual clinical signs.[12,13] The complexion, especially the skin of the face and anterior neck, of patients treated over a long period became ruddy and flushed. Injection of the conjuctiva was present. Although the flushing and ruddiness would disappear in a few days after discontinuing androgen therapy, the hemoglobin remained elevated for longer periods of time. The high values, however, were not maintained indefinitely.

In one patient who received ethinyl estradiol and then testosterone propionate there was an increase in the hemoglobin from 6.4 to 18.0 gm., and an increase of red blood cells from 2.4 to 5.9 million per cu. mm. This was accompanied by improvement of the osseous lesions.

COMMENTS

Significant biochemical and metabolic changes have been observed during administration of sex steroid hormones in patients with metastatic carcinoma of the breast. The alterations noted further clarify the mechanisms of tumor regression occurring during hormone therapy, and allow selection of specific biochemical determinations as therapeutic guides in the management of such therapy.

Pathologic examination of tumor masses undergoing regression during hormone therapy revealed the immediate cause of softening of the tumors to be a loosening of the connective tissue surrounding tumor cells. Collagenous stromal bands were reduced to narrow tangled fibrous strands separated by wide interspaces. The widened interspaces contained altered cancer cells, infiltrating plasma cells and lymphocytes, and a tangle of delicate fibrils. Concurrently with this loosening of stroma, tumor cells degenerated and disappeared. The process of loosening reversed and fibrosis of the area occurred with accumulation of mature collagen. During this later process elastic tissue fibers were deposited over large areas. Osseous lesions undergoing regression further demonstrate the formation of new bone. The histologically more differentiated tumors and the clinically more slowly growing tumors appeared more responsive to hormone therapy.[14,15]

X-ray examinations of osseous metastases initially revealed osteolytic lesions in most instances. In patients with a clinically slower progressing tumor a small rim of increased density is noted about the osteolytic (osteoclastic) lesions and appears to represent an attempt at bone repair (osteoblastic activity). Occasionally, the lesions are largely of increased density and are referred to as osteoblastic metastases. These appear to be slower growing lesions, sufficient time elapsing to allow an intense reparative reaction to occur about and within the metastasis. In patients in whom there is no improvement during hormone therapy the osteolytic lesions continue to increase in size and extent. In those patients demonstrating improvement as a result of therapy, the metastatic lesions become dense (osteoblastic repair with calcifica-

tion) or the bones appear to be normal in structure. Relative differences in the degree of repair are noted in patients with less striking improvement; in many, both continued progress of osteolytic metastases and the formation of osteoblastic (repaired) lesions are present.

Such reparative or progressive processes noted on x-ray and pathologic examination were correlated with the biochemical determinations observed.

Calcium. It is recognized that the processes of bone formation (osteoblastic activity) and bone reabsorption (osteoclastic activity) occur simultaneously in the normal individual. The presence of osseous metastases disrupts this normal pattern in the areas of the tumor, and this disruption is in part manifested by alterations of calcium metabolism. Varying degrees of elevation of the serum and urinary calcium have been observed to occur in patients with cancer in association with osseous metastases. Such increases are found particularly in patients with breast cancer, in which the lesions are predominantly osteolytic.[17] In patients with extensive metastases or a rapidly growing tumor the bone destruction is so great as to result in excessive release of calcium from the bone and marked increase in serum calcium levels occur. The "spontaneous hypercalcemia syndrome" is invariably accompanied by characteristic signs and symptoms. Intermediate variations in the clinical and chemical findings are more common, and the symptoms and signs are usually subtle or absent. The diagnosis in such instances is as a rule made only by laboratory studies. In patients in whom this syndrome is severe or long-standing, calcium deposition may occur in various tissues and in the renal tubules, resulting in uremia and at times irreversible renal damage. It was in this respect that non-protein nitrogen determinations were of value in observing the patient's course.

In the present study untreated patients with osteolytic metastases had a normal or elevated serum calcium. Low values were not encountered. Spontaneous hypercalcemia occurred in 12 per cent of patients demonstrating osseous metastases by x-ray examination. In two patients hypercalcemia preceded the detection of osseous lesions by x-ray examination. It would appear that hypercalcemia in the untreated patient with advanced cancer of the breast is indicative of osseous metastases although not demonstrable on roentgenologic examination, and that the presence of active breast cancer without osseous involvement has no direct effect on the calcium metabolism.

In no patient with hypercalcemia was there elevation of the serum protein; hence, the increase in serum calcium apparently was not due to an increase in the protein-bound fraction.

The presence of breast cancer elicits a primary attempt at repair by the host,[14,15] and bone at the site of metastases may exhibit increased osteoblastic activity. However, if the rate of cancer growth and bone destruction is rapid, osteolysis may be sufficient to cause hypercalcemia.

Observation of the serum calcium during steroid hormone therapy aided in evaluating the effect of such therapy on the tumor. In patients with initial "spontaneous" hypercalcemia the serum calcium decreased to normal values. It is presumed that excessive osteolysis of bone due to tumor was inhibited. The regression of tumor growth apparently resulted in decreased bone breakdown, increased osteoblastic activity, new bone formation, increased calcium deposition, and hence a decrease in serum calcium and urinary calcium excretion. The presence of hypercalcemia is not a contraindication to the institution of steroid hormone therapy. In some instances the decrease of serum calcium may be a direct effect of the hormone on calcium metabolism, as recorded in other bone disease. No hypocalcemia was detected.

A condition referred to as the "induced hypercalcemia syndrome" occurred in a limited number of patients.[5] This syndrome, manifested by drowsiness, anorexia, nausea, vomiting, dehydration, stupor and coma, may terminate fatally. Close observation of the patient receiving hormone therapy is necessary so that corrective therapy can be instituted early. In our experience no patients clinically free of skeletal metastases have developed hypercalcemia during hormonal therapy. Estrogenic hormones seldom produced hypercalcemia in patients with osseous metastatic lesions whereas the occurrence of this syndrome during androgenic therapy, although infrequent, was more common. With the use of either hormone the development of the symptoms described in patients with demonstrable osseous metastases from breast cancer should be considered as presumptive evidence of impending hypercalcemia, even though the laboratory findings are within normal limits.

A clear explanation of this phenomenon is not

available. Investigations suggest several possibilities: (1) an initial acceleration of tumor growth with rapid bone destruction resulting in hypercalcemia and hypercalciuria, (2) interference by the hormones of the spontaneous attempt at bone repair, or breakdown of tumor barriers, (3) a direct effect of the hormones on calcium metabolism, (4) a similarity in the alterations to that of parathyroid hormone with an increase of urinary phosphorus, hypophosphatemia, hypercalcemia and hypercalcuria,[4,5] and (5) immobilization.

Phosphorus. The estimation of serum phosphorus was important initially only in reference to later hormone therapy and evaluation of progress of the disease, rather than as a diagnostic aid. The serum phosphorus initially was elevated above 6 mg. per cent in only two patients with osseous metastases. Neither had hypercalcemia or renal disease. With excessive bone destruction an increased serum phosphorus might be expected, usually in association with a high serum calcium. Hypophosphatemia was present in twenty-three patients. Secondary parathyroid hyperplasia might conceivably be present in those patients with osseous metastases, but the low levels in patients without demonstrable osseous lesions are not explained. A low serum phosphorus has been detected previously in patients with advanced skeletal metastases or cachexia.[17]

A decrease of serum phosphorus of more than 1.0 mg. per cent occurred in fifty-four patients (62.8 per cent) during hormone therapy. As a rule this decrease was maintained as long as the tumor was controlled or the hormone administered. The fall in serum phosphorus during endocrine therapy might be due to a reduction in the rate of bone destruction. This may be true with testosterone therapy. However, twenty-seven patients receiving estrogenic hormone and in whom no osseous metastases were demonstrated also had a reduction of serum phosphorus; patients without osseous metastases manifested a decrease in serum phosphorus as frequently as those with osseous lesions. The fall in serum phosphorus does not appear to be solely an effect of the treatment on the metastatic tumor, since in patients with postmenopausal osteoporosis the serum phosphorus fell in almost all instances when estrogens were administered.[18] There was no correlation between the fall in serum phosphorus and the initial clinical response of the tumor during hormone therapy. Furthermore, no significant correlation could be made between the decrease in serum phosphorus and the development of fluid retention. In the absence of similar decrease of other chemical components it would appear that this phenomenon is not the result of increased body fluid and resultant hemodilution.

In one metabolic study of a patient with extensive osseous metastases the alterations noted were similar to those that occur with the administration of parathyroid hormone; that is, an initial increase in urinary phosphorus, fall in serum phosphorus, rise in serum calcium and an increase in urinary calcium.[6,8] This patient demonstrated the "induced hypercalcemic syndrome."

It has been reported that during the administration of testosterone or estrogenic hormones to patients with active gigantism and active acromegaly, the elevated serum phosphorus decreased and the phosphorus balance became more positive. It was also noted that in the growing child there is an elevation of serum phosphorus which falls to the adult level shortly after the onset of puberty. It has been proposed that serum phosphorus may be an index of pituitary growth hormone production in human subjects, and that estrogen or testosterone inhibit the production of pituitary growth hormone.[19] Postulation of a similar relation in breast cancer is possible and warrants further intensive investigation.

In some patients whose tumor regressed during hormone therapy a rise in serum phosphorus occurred just prior to and during an exacerbation or progression of the tumor despite continued hormone therapy. It has been possible to predict a forthcoming "escape" of the tumor from hormone therapy and subsequent progression of the disease by this increase in serum phosphorus which may not be accompanied by hypercalcemia.

Alkaline Phosphatase. It is clear that abnormally increased osteoblastic cell activity is accompanied by an elevated alkaline phosphatase of the circulating blood, roughly proportional to the extent and intensity of the attempt at ossification.[16] When destructive (osteolytic) and reparative (osteoblastic) processes occur simultaneously, as in osseous metastases, the constructive process alone is reflected in the alkaline phosphatase activity of the blood serum. In tumors without bone or liver involvement the serum phosphatase activity is usually

normal.[16] The elevation of serum alkaline phosphatase in liver disease is a well defined entity.

The alterations of serum alkaline phosphatase are a manifestation of both hepatic and osseous changes. The distinction between these factors is necessary in interpreting the initial values obtained, as well as in evaluating later changes during hormone therapy. The alkaline phosphatase is elevated during the processes of new bone formation or increased osteoblastic activity. This activity is stimulated in part by the ordinary strain or stress of mobilization. In patients with rapid destruction of bone due to breast cancer metastases an unsuccessful attempt at "spontaneous" bone repair may occur. If the disease is slower growing, the normal defense mechanism of the body may attempt bone repair (increased osteoblastic activity) about the site of the invading lesion, resulting in an increased serum alkaline phosphatase and a rim of increased density about the lesion on x-ray examination. A similar change may occur in the presence of a pathologic fracture with repair. Of the patients with osseous metastases, nineteen (40.4 per cent) had serum alkaline phosphatase levels above 6.0 B.U. Only one of these had liver metastases. In those without demonstrable liver metastases, the values did not exceed 15 B.U., nor did they increase as rapidly as in patients with liver metastases. Since breast carcinomas with a lower degree of malignancy appear to respond favorably to hormone therapy, those patients with elevated serum alkaline phosphatase without liver involvement might be expected to do well. Outstanding regression of bone lesions occurred in twelve of the nineteen patients with elevated alkaline phosphatase levels. In two patients with Paget's disease of the bone, the serum alkaline phosphatase was higher (30 and 24 B.U.) than that usually seen in patients with osseous metastases.

In twenty patients without osseous metastases or Paget's disease, the serum alkaline phosphatase was elevated over 6 B.U. Six of these patients manifested osseous metastases within a few weeks. In seven patients the increase in alkaline phosphatase activity was due to liver metastases that were already present or were manifest clinically soon thereafter; the degree of elevation being from 10 to 36 B.U. The alkaline phosphatase appeared to be the most sensitive guide for the detection of hepatic metastases. Elevation of the serum alkaline phosphatase has been described in patients with liver metastases even when there was no elevation of the serum bilirubin.[20–22] It has been found that the extent of elevation and the frequency of these elevated values increase with the extent of the metastatic involvement of the liver.[22,23] Since the tumor cells contain less than normal amounts of alkaline phosphatase, it is unlikely that these cells are the source of the enzyme.[24]

It is apparent that in patients with advanced cancer of the breast an elevation of the serum phosphatase may be indicative of hepatic metastases or osseous metastases even though lesions are not demonstrable on roentgen examination.

During hormone therapy of patients with osseous metastases but without hepatic metastases, an increase of the serum alkaline phosphatase was observed in 51.1 per cent. All the patients with osseous metastases who responded favorably to hormone therapy had an associated rise in the serum alkaline phosphatase. There is no evidence that this was due to direct stimulation of osteoblastic activity. Previous studies in patients with postmenopausal osteoporosis demonstrated no change in the serum alkaline phosphatase during estrogenic or androgenic hormone therapy.[25] This suggests that as a result of the decrease in size of the tumor attempts at bone repair (osteoblastic activity) has occurred. Furthermore, stimulation of normal tissue repair may occur, similar to that noted in the primary breast tumor undergoing regression during hormone therapy.[14,15] In patients with widespread carcinoma of the breast without osseous lesions demonstrable by x-ray, the presence of such metastases may be presumed from the increase in serum alkaline phosphatase during hormone therapy. Relief of pain and appearance of osteoblastic lesions on x-ray are further evidence of the presence of such lesions.

In eleven patients no reason could be assigned for the decrease of alkaline phosphatase that occurred. It may be postulated that the hormone agent employed impaired the normal reparative osteoblastic activity by stimulation of tumor growth.

During hormone therapy progression of hepatic metastases was associated with a steady increase in serum alkaline phosphatase. In a few instances, when regression of the liver metastases occurred, the alkaline phosphatase decreased to normal values. If, however, osseous metastatic

improvement occurred concurrently, the alkaline phosphatase decreased but to a level above normal; the persistence above normal being the result of osteoblastic activity.

An explanation for the elevation of alkaline phosphatase due to liver metastases has been postulated.[23] As the hepatic tumor mass enlarges, it compresses and distorts the surrounding hepatic tissue. The intrahepatic bile ducts near the mass are obstructed, leading to a biliary obstruction similar to that of ligation of minor extrahepatic ducts in animals, and the serum alkaline phosphatase increases. The greater the number of tumor nodules, the greater the number of ducts obstructed; hence the higher the serum alkaline phosphatase. When a sufficient number of ducts are blocked, jaundice occurs. Consequently, when hormonal therapy results in the reduction of tumor size, the biliary ducts are no longer obstructed and the alkaline phosphatase decreases.

In the present investigation, as in previous studies, it would appear that the serum alkaline phosphatase determination was the only liver function test employed that was of significant value in detecting liver metastases even in early stages. The presence of osseous lesions must be considered in evaluating this method of detecting impairment of liver function.

Acid Phosphatase. An increase in serum acid phosphatase was noted in patients with osseous metastases. Two patients demonstrated an increase in the acid phosphatase during hormone therapy, eventually associated with an "induced hypercalcemic syndrome."

Slight increases in serum acid phosphatase in hyperparathyroidism have been observed to fall promptly to normal values following removal of parathyroid adenomas. Since bone contains small amounts of acid phosphatase, it has been suggested that the "bone acid phosphatase" may be the origin of small increases in serum acid phosphatase in hyperparathyroidism.[10] Similarly, it would appear that in patients with osseous metastases from breast cancer the increase in serum acid phosphatase during hormone therapy was due to an increased rate of bone destruction and mobilization of "bone acid phosphatase." The eventual development of hypercalcemia further supports this premise. The hormone administered presumably had no effect on the normal rate of progression of the disease, or stimulated the rate of tumor growth increasing the rate of bone destruction.

Liver Function Tests. Liver function studies were conducted to observe alterations that might occur as a result of intensive hormone therapy. No significant alteration of the serum total protein occurred. The changes noted were those anticipated in patients with extensive debilitating disease. The use of this test as a guide to hormone therapy of breast cancer does not appear to be of value. The cephalin flocculation test, although affected by the presence of liver metastases, did not appear to be of diagnostic aid in ascertaining the presence of metastases until these lesions were easily demonstrable clinically. Despite the presence of extensive liver metastases, the van den Bergh test remained within normal limits, nor did hormonal therapy alter the test. The serum bilirubin became elevated only when extensive liver metastases were easily demonstrable clinically, and was not a useful guide for hormone therapy. The bilirubinemia that occurred during the "induced hypercalcemia syndrome" is of interest and might possibly be explained by red cell hemolysis or to increased viscosity of the blood.[26] The serum alkaline phosphatase, as already indicated, was the only liver function test employed that was of significant value in all stages of liver metastases.

Hematopoietic System Alterations. It was apparent that a decrease of erythroid activity during estrogenic hormone therapy was associated with progression of the cancer. The extensive involvement of bone marrow and/or decrease in the nutritional status of the patient were prominent features. The increase in erythroid activity, conversely, was associated with improvement in nutritional status and/or regression of osseous metastases, resulting in more normal marrow activity.

The dissociation between the clinical course of the tumor and the increased erythroid activity during androgenic hormone therapy suggests that androgenic hormones may be a stimulus to the marrow. That this hormone may play a role in folic acid metabolism has also been suggested.[27]

Biochemical Aids for the Management of Hormone Therapy. By analysis of the biochemical alterations occurring during hormone therapy, a method for management of hormone therapy in patients with advanced breast cancer can be outlined. It would appear that certain chemical determinations are necessary as an aid in establishing the presence and site of metastases

and in evaluating the tumor response to therapy. Initially, the following procedures are carried out: serum calcium and phosphorus, serum alkaline phosphatase, BUN or NPN, and hemoglobin. At the end of one week a serum calcium is obtained for detection of early hypercalcemia. Thereafter, these procedures are repeated at intervals of three to six weeks. After four months further studies are obtained as indicated. In patients receiving long term hormone therapy, or in whom response to therapy has been favorable, determination of the serum calcium, phosphorus, alkaline phosphatase and hemoglobin every two to four months aids in anticipating new changes in tumor growth. In conjunction with these studies, appropriate x-ray examinations are conducted. By such procedures evaluation of the progress of the tumor can be made, and improved patient care thereby attained.

SUMMARY

1. Determinations of serum calcium and alkaline phosphatase are of value in establishing the presence of osseous and hepatic metastases in advanced breast cancer.

2. Steroid hormone therapy for advanced breast carcinoma produces biochemical alterations in the blood which aid in evaluation of the tumor response to such therapy.

3. The serum calcium, phosphorus, nonprotein nitrogen and alkaline phosphatase appear to be important guides in long term hormone therapy of advanced breast cancer.

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The Pathogenesis of Anemia in Patients with Carcinoma*

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RECENT investigation of the development of anemia in association with lymphomas and leukemia has placed emphasis on the role of hemolysis. Ross,[1] Tedeschi,[2] Berlin[3] and others have independently described the frequency of accelerated red cell destruction in such patients. A small percentage of these patients shows overt hemolytic phenomena but in the majority more sensitive methods are required to demonstrate this anomaly.

In a preliminary report from this laboratory[4] use of the Ashby technic for study of the red cell life span demonstrated a considerable acceleration of destruction of the transfused normal cells in a small number of patients with carcinoma. In order to study this problem more fully, attention in the present investigation is directed toward two factors: (1) The role of hemolysis as measured by routine clinicopathologic tests and the Ashby technic, and (2) the rate of erythropoiesis. In the latter study interpretation of the marrow aspirate was combined with evaluation of the ferrokinetics of erythropoiesis, utilizing the radioisotope Fe^{59}.

MATERIALS AND METHODS

Forty-five patients with many types of biopsy-proved carcinoma were chosen. In these patients all studies failed to demonstrate any overt cause for anemia such as blood loss. They were not receiving and had not recently received any therapy which might lead to anemia or might depress marrow function, such as radiotherapy, tumor chemotherapy or transfusion. In every instance either skeletal x-ray survey, marrow aspiration and/or ultimate necropsy showed that the disease was widely disseminated. The types of epithelial neoplasm included carcinoma of the lung, breast, stomach, esophagus, prostate, kidney, ovary and thyroid.

TABLE I
CLASSIFICATION OF HEMOLYSIS

Procedure	Normal	Slight	Moderate	Marked
Mechanical fragility (%)	<5	5–10	>10	>10
Osmotic fragility	0.45–0.50	>0.50	>0.50	>0.50
Reticulocyte count (%)	<3	3.1–5	5.1–10	>10
Fecal urobilinogen (E.U./100 gm.)	<200	200–350	350–600	>600
Urine urobilinogen	1/10–1/160	1/20–1/40	>1/40	>1/40
Coombs' test (direct)	Negative	Negative	Positive	Positive
Bilirubin-delayed (mg. %)	0.1–1.0	1–2	2–5	>5

Anemia was present in more than 90 per cent of the patients at the outset of the investigation and eventually in all cases.

The clinicopathologic studies are those routinely used (Table I) and are fully described in a previous report.[4] The Ashby technic employed was that described by Kaplan and Zuelzer.[5] Patients were so selected that the great majority had never had previous transfusions.

Plasma iron and iron-binding capacity were determined by the Kitzes[8] and Cartwright[9] method. The plasma and red cell iron turnover was calculated by the Hennessy-Huff equation.[10] Radioactive iron-59 was obtained from the Atomic Energy Commission as ferric chloride in dilute hydrochloric acid. The amount of

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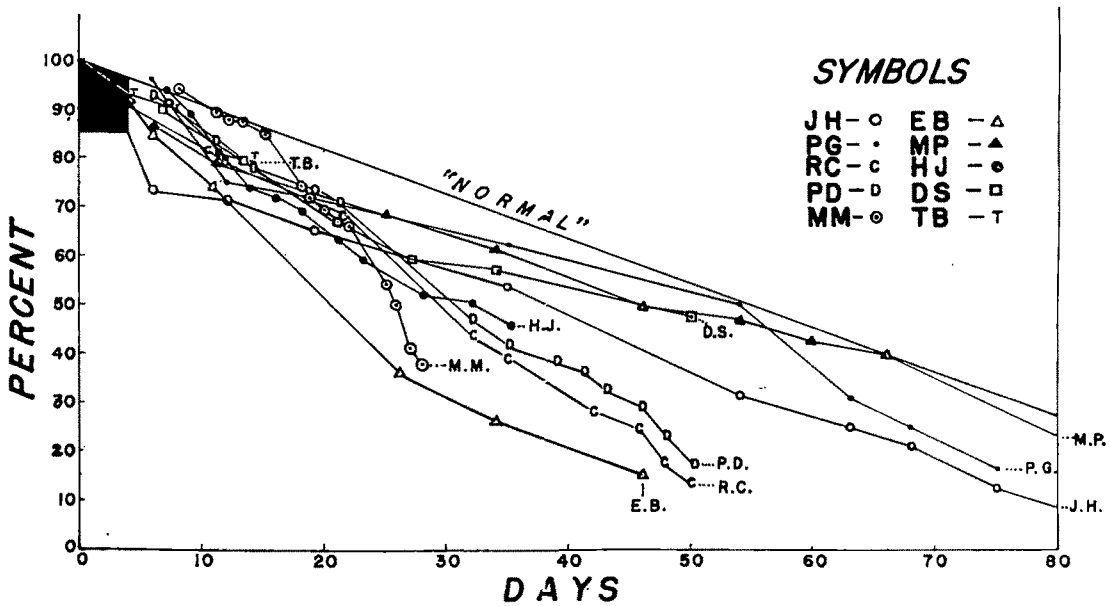


FIG. 1. Ashby-type curves in ten patients with carcinoma.

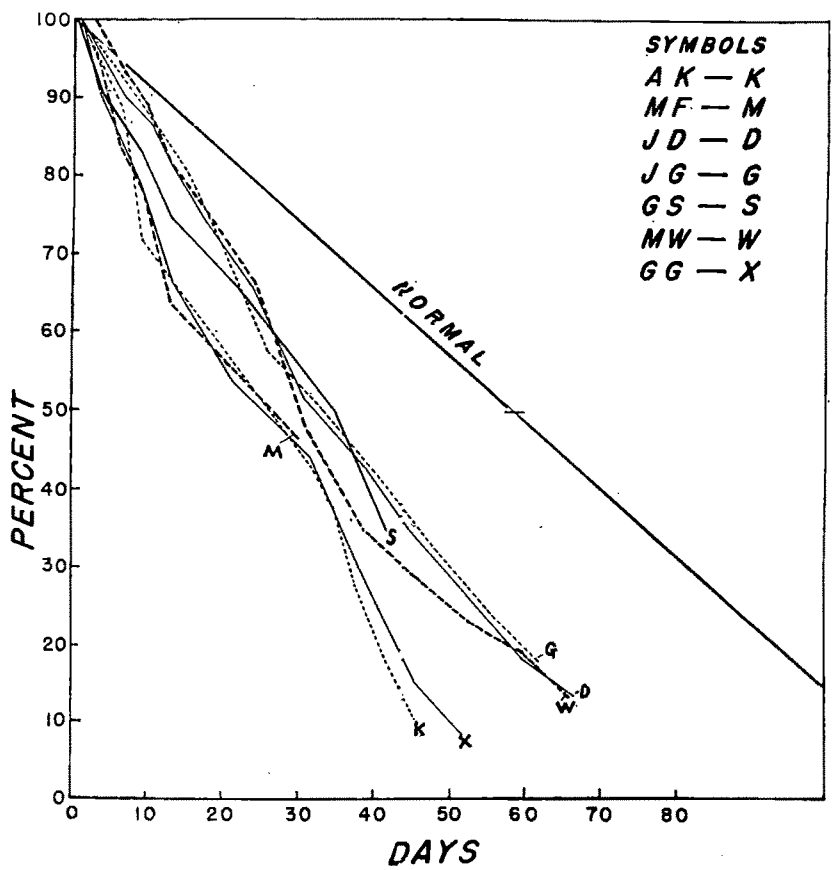


FIG. 2. Ashby-type curves in seven patients with carcinoma.

radioactivity injected was approximately 5–10 microcuries/patient.

RESULTS

Clinicopathologic Observations. Twenty-six patients with carcinoma were studied by the usual multiple clinicopathologic tests. A moderate increase in hemolysis was found in one patient (4 per cent), a slight increase in hemolysis in fourteen (54 per cent) and no significant change in eleven (42 per cent). Further details are given in Table II.

Ashby Data. Ashby-type studies, using the blood of healthy donors were made nineteen times in seventeen patients with a variety of carcinomas, and eleven times in ten patients with neoplasms other than carcinomas including a variety of blood dyscrasias. In the patients with carcinoma (Figs. 1 and 2) fifteen of the nineteen studies showed a moderate to marked increase in the rate of destruction of the transfused cells. In all of these fifteen instances 50 per cent of the transfused cells were destroyed in thirty-five days or less. Three of the patients showed a slight increase in the rate of destruction of the transfused cells and in one patient the Ashby curve was approximately normal. In two instances (Fig. 3) repeat studies revealed further increase in the rate of hemolysis in association with progression of the disease.

For comparison, data on ten patients with neoplastic disease other than carcinoma (such as chronic myeloid leukemia, chronic lymphatic leukemia, agnogenic myeloid metaplasia, multiple myeloma, Hodgkin's disease and fibrosarcoma) were summarized in Figure 4. In all eleven instances in which studies were made

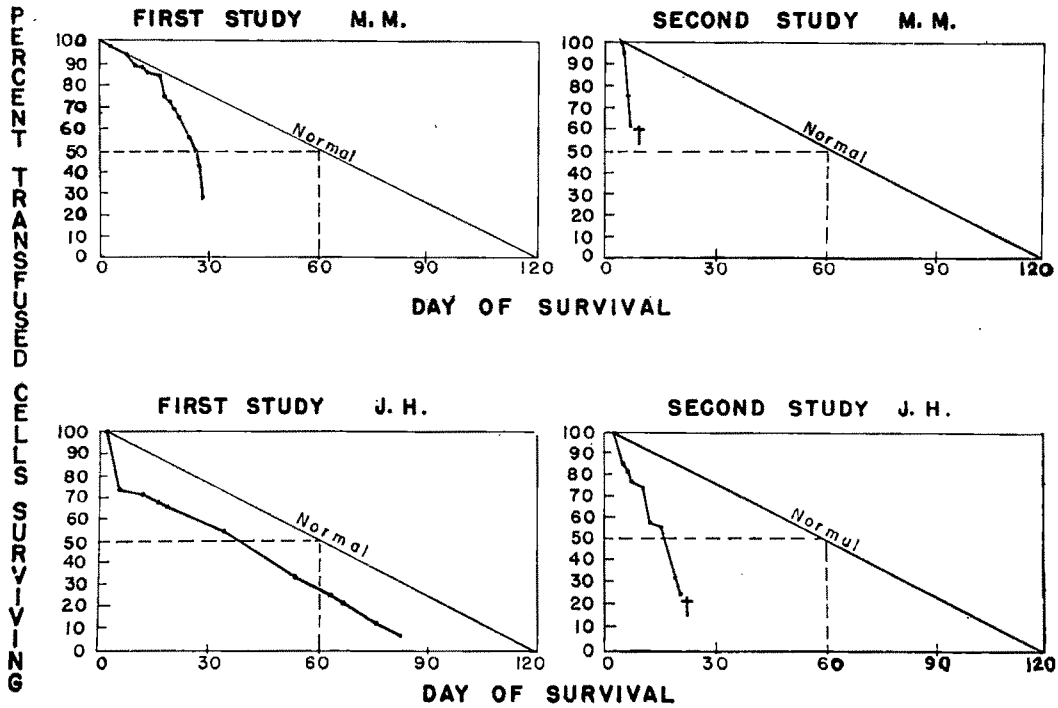


FIG. 3. Characteristic Ashby-type curves in two patients with neoplastic disease and repeat studies when the disease progressed.

TABLE II

DEGREE OF HEMOLYSIS IN TWENTY-SIX PATIENTS WITH CARCINOMA (ROUTINE STUDIES)

Estimated Degree of Hemolysis	Total	Clinicopathologic Findings: Elevation above Normal Values				
		Reticulocytes	Fecal Urobilinogen	Urine Urobilinogen	Indirect Bilirubin	Mechanical Fragility
None.....	11(42%)	..	..	..	..	..
Slight....	14(54%)	12	2	5	..	4
Moderate.	1(4%)	1	..	..	..	..
Total...	26(100%)	..	..	..	..	..

moderate to marked acceleration of the rate of destruction of donor cells was found. The "shift to the left," or more extreme shortening of the life span, was greater in this group than in the patients with carcinoma.

plasma iron level, as found in thirty-one of our forty-five patients, in association with depression of plasma iron-binding globulin. Therefore the saturation percentage may show normal results, as occurred in fourteen of our forty-five patients.

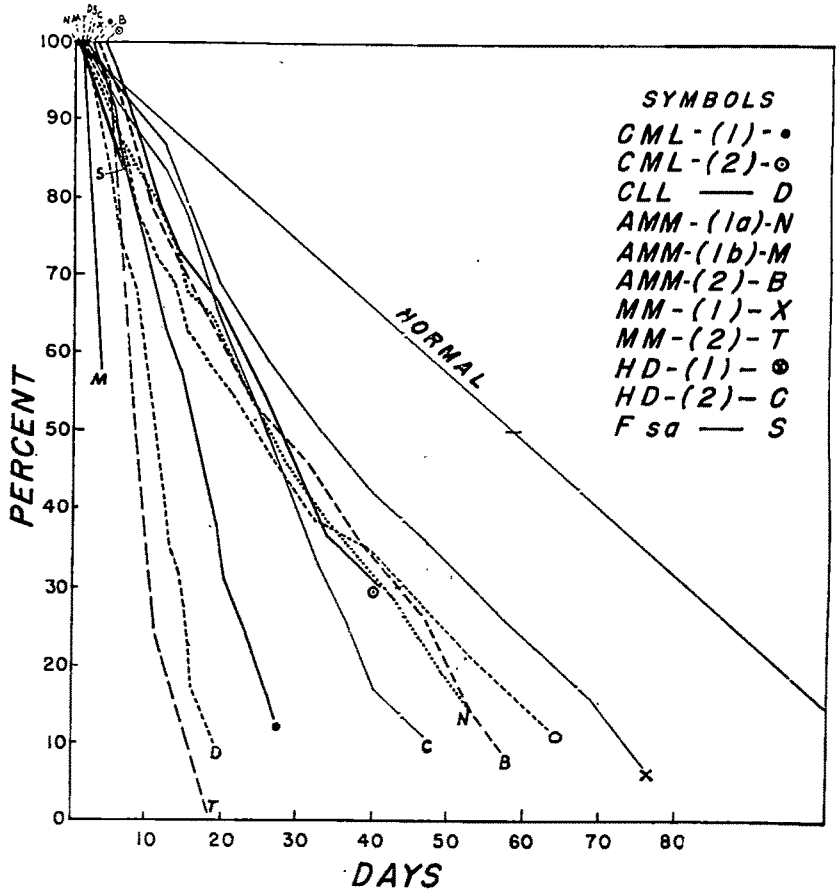


FIG. 4. Ashby-type curves in ten patients with neoplasms other than carcinoma.

Marrow Activity. In forty-five patients bone aspirates gave positive results for tumor cells. When the degree of cellularity or marrow activity was estimated, only four (8 per cent) exhibited decreased cellularity, thirty-five (78 per cent) gave normal or slightly decreased results and six (14 per cent) were found to have increased in the degree of cellularity.

Studies of Iron Metabolism. Forty-five patients with documented carcinoma were studied using Fe^{59} combined with iron-binding globulin (Cohn-fraction IV-7*). In this type of patient the most frequent observation is a depression of

Ferrokinetic studies are summarized in Tables III and IV. As can be seen, the half-time value for the removal of plasma Fe^{59} following intravenous injection, normally reached in sixty to 120 minutes, was shortened in nineteen of the patients and normal in sixteen. In only ten of the forty-five patients, or 22 per cent, was there prolongation of this half-time value. When attention is directed to plasma iron turnover in milligrams per day, it will be noted that many of the patients (twenty-one of forty-five, or 47 per cent) gave normal values. Red cell iron turnover data generally gave either normal or increased results. Both plasma iron removal rates and red cell iron renewal rates frequently increased. Calculation of red cell iron

* This fraction was supplied by Dr. Joyce M. Flynn, University Laboratory of Physical Chemistry, Harvard University, Boston, Mass.

utilization after fourteen days indicated that 69 per cent of the patients had normal values.

COMMENTS

Routine clinicopathologic tests gave little indications of increased hemolysis. The only abnormalities found were: (1) a slight elevation of the reticulocyte level in one-half of these patients; (2) an elevation of fecal urobilinogen in two patients, and (3) a mild increase in the mechanical fragility in urinary urobilinogen in one-fourth of the patients. The vast majority of patients showed either no evidence or very slight evidence of the increased hemolysis revealed by more refined studies.

A Coombs cross matched, Ashby-type transfusion was given in thirty instances in the course of this study. Nineteen of the studies in patients with advanced carcinoma revealed a moderate to marked increase in the rate of destruction of the transfused normal cells. Eleven studies in patients with non-carcinomatous neoplastic

TABLE III

SUMMARY OF DATA ON IRON METABOLISM IN FORTY-FIVE PATIENTS WITH ADVANCED CARCINOMA

Case No.	Patient	Plasma Fe[1]	Saturation[1] (%)	Fe Half-time[2a] (min.)	Plasma Fe Turnover[2b]		Red Cell Fe Turnover[2c]		Fraction of Plasma Fe Removed /hr.[2d]	Fraction of RBC Fe Renewed /day[2c]	Red Cell Mass (cc.)[3]	Plasma Volume (cc.)[3]	Per cent of Fe^{59} Utilized by RBC in 14 days[4]
					Mg./day	Mg./Kg./day	Mg./day	Mg./Kg./day					
1	A. A.	125	46	224	23.3	0.35	.....	....	0.25	.....	2052	3092	6
2	E. B.	91	39	106	27.6	0.5	27.6	0.5	0.37	0.02	1386	3400	26
3	E. D.	60	24	60	25.0	0.38	22.5	0.34	0.62	0.014	1680	2800	54
4	B. D.	100	27	150	15.4	0.31	9.2	0.19	0.28	0.014	660	2273	91
5	T. G.	76	54	68	23.5	0.52	5.4	0.12	0.70	0.009	597	1843	23
6	E. F.	49	17	49	31.0	0.35	46.4	0.53	0.68	0.011	3290	3860	42
7	C. H.	67	34	70	23.5	0.35	23.0	0.34	0.52	0.015	1508	2800	58
8	A. K.	89	27	117	21.1	0.30	25.3	0.35	0.31	0.009	2931	3306	41
9	F. L.	83	39	132	18 5	0.50	29.6	0.80	0.46	0.017	1705	2001	89
10	S. M.	90	38	75	47 8	.....	52.6	....	0.96	0.020	2600	2300	41
11	O. McK.	45	36	59	18.0	0.27	20.5	0.31	0.55	0.010	2010	3015	57
12	M. P.	50	24	70	21.4	0.26	25.7	0.31	0.58	0.010	2490	3410	50
13	R. S.	36	17	176	13.7	.....	10.7	....	0.86	0.013	828	1842	36
14	J. S.	44	28	88	31.7	.....	25.0	....	1.36	0.025	1000	2200	76
15	D. A.	43	22	38	31.2	0.46	61.5	0.90	0.94	0.309	1994	3278	92
16	B. R.	97	49	204	24.3	.....	3.4	....	0.44	0.005	705	2500	(11 D—19%)
17	S. B.	69	42	30	49.4	1.02	31.2	0.65	1.54	0.014	753	1935	58
18	A. B.	133	60	135	28.8	0.8	5.8	0.16	0.44	0.008	720	2000	25
19	L. L.	58	38	56	21.4	0.50	13.1	0.30	0.59	0.018	750	2570	(6 D—81%)
20	T. S.	45	29	42	26.4	0.49	62.1	1.17	0.96	0.036	1701	2552	107
21	F. W.	55	35	64	32.6	.....	22.9	....	0.62	0.013	1783	3973	32
22	J. R.	103	43	47	47.0	0.78	37.6	0.62	0.73	0.026	1462	2600	(13 D—54%)
23	D. S.	51	31	40	43.2	0.864	40.6	0.81	0.87	0.025	1638	2912	57
24	J. B.	110	61	112	26.2	0.51	6.6	0.13	0.43	0.009	725	1909	35
25	B. L.	107	53	132	14.2	0.23	12.6	0.20	0.21	0.008	1618	2640	58
26	S. F.	81	33	83	16.6	0.28	13.3	0.22	0.39	0.011	1180	2190	62
27	J. H.	35	11	122	8.9	.....	6.6	....	0.33	0.003	1938	2672	37
28	L. R.	85	32	150	9.4	0.18	4.7	0.09	0.21	0.003	1467	2200	54
29	P. G.	60	28	104	10.6	0.18	4.8	0.08	0.31	0.006	764	1700	57
30	M. M.	105	35	180	9.8	0.197	3.8	0.08	0.16	0.004	936	2417	(13 D—30%)
31	E. H.	75	29	146	195.4	.....	.....	.....	2.46	.....	1717	4416	.....
32	M. W.	40	15	50	4.3	0.08	.....	.....	0.17	.....	....	2592	61
33	A. L.	63	21	44	43.6	0.51	17.7	0.21	0.9	0.033	553	3132	72
34	G. C.	53	19	52	22.2	0.48	34.2	0.81	0.67	0.023	1468	2610	71
35	T. B.	114	36	110	17.5	0.33	10.9	0.20	0.33	0.010	1041	1933	(13 D—70%)
36	G. G.	93	30	86	53.3	0.80	42.1	0.63	0.70	0.025	1671	3394	(13 D—30%)
37	F. H.	63	21	55	30.5	0.47	.....	....	0.75	.....	....	2684	83
38	R. M.	124	40	38	94.1	1.88	177.8	3.55	1.38	0.14	1233	2290	83
39	E. D.	75	20	57	24.	0.67	.....	....	0.65	.....	....	2044	102
40	F. P.	60	27	64	21.8	0.31	28.6	0.40	0.51	0.012	2238	2967	50
41	C. G.	28	9	42	9.1	0.15	.....	....	0.42	.....	....	3222	62
42	B. T.	40	11	58	13.3	0.26	.....	....	0.60	.....	....	2302	(6 D—80%)
43	S. G.	73	19	58	31.7	0.63	.....	....	0.72	.....	....	2511	66
44	E. D.	37	10	51	14.5	0.29	.....	....	0.66	.....	....	2105	(13 D—63%)
45	J. G.	52	15	80	19.7	0.28	.....	....	0.55	.....	....	2843	(13 D—59%)

[1] Average of two or more determinations. Normal for plasma Fe/total iron binding globulin: 90–130 μg. per cent/260–350 μg. per cent. Normal plasma Fe saturation: 30 to 40 per cent.

[2] Normal values in this laboratory: (*a*) intravenous plasma Fe ½ time clearance: 60–120 min.; (*b*) plasma Fe turnover: 20–37 mg./day; (*c*) RBC Fe turnover: 14–38 mg./day; (*d*) fractions of plasma Fe removed/hr: 0.33–0.50; (*e*) fraction of RBC Fe renewed/day: 0.0072–0.0107.

[3] Calculated on basis of Fe^{59} studies—normal total blood volume approximately 77 cc./kg.

[4] Per cent of Fe^{59} administered intravenously appearing in RBC's 14 days after injection—normal 70–100 per cent.

disease, including a variety of blood dyscrasias, showed a further increase in the rate of destruction of normal donor cells. Thus the patients with carcinoma fell between this latter group and the normal.

the theory that erythropoiesis is normal or increased in the "average patient" with carcinoma. Since anemia develops in spite of normal or increased marrow function, apparently the demonstrated increase in hemolytic activity is

TABLE IV

IRON METABOLISM STUDIES IN FORTY-FIVE PATIENTS WITH CARCINOMA

Activity	Plasma Iron		Plasma Disappearance (rate)	Plasma Iron Turnover	Plasma Iron Removal (rate)	Red Cell Iron Turnover	Red Cell Iron Renewal (rate)	Red Cell Iron Utilization (14 days)
	Levels	Saturation (%)						
Decreased...	31	23	19	16	9	14	5	14 (31%)
Normal.....	14	14	16	21	9	14	8	31 (69%)
Increased...	0	8	10	8	27	7	22	.....
Total.....	45	45	45	45	45	35*	35*	45 (100%)

* Complete data not available in ten patients.

Marrow failure and/or replacement have been blamed for the anemia of patients with disseminated carcinoma. Our observations seem to justify a different interpretation. Normal cellularity was found in the great majority of instances even when tumor cells were present. In view of the limitation of morphologic technics attention was turned to the dynamics of iron metabolism.

The rate at which intravenously administered iron disappears from the plasma has been thought by some observers[11] to reflect the state of erythropoiesis in the individual. In these patients with carcinoma a great majority did have either increased or normal disappearance rates. These observations, therefore, would indicate normal marrow activity. However, since the iron disappearance rate may not necessarily reflect the status of erythropoiesis in patients with malignant disease, two other studies were performed. The first was the measurement of the red cell iron renewal rate per day. The second was the estimation of the percentage of Fe^{59} utilized in red cell production at a given time (for example fourteen days) following intravenous injection. These studies yielded further support to the theory that erythropoiesis is either normal or even increased in the majority of these patients. Thus it would seem that the direct and indirect evidence afforded by ferrokinetic studies, as well as that of the marrow morphology, support greater than the marrow response in these patients. Clinical correlation with this theory is found in the common experience that frequent transfusions may be unsuccessful in maintaining adequate hemoglobin levels in advanced neoplastic conditions.

What factors are present in the plasma or the red cells of these patients with advanced carcinoma that lead to this increase of hemolysis provides a separate and complex problem in the metabolism of neoplastic disease which deserves further study.

SUMMARY

1. Forty-five patients with biopsy-proven, advanced carcinomas were selected for study of the pathogenesis of previously unexplained anemia.

2. Routine clinicopathologic tests revealed no significant increase in hemolytic activity.

3. The Ashby technic did demonstrate considerably increased rates of destruction in fifteen instances in patients with carcinoma, and in eleven instances in patients with non-carcinomatous neoplasm.

4. In the majority of patients studied normal marrow activity was found even in the face of marrow invasion by tumor cells.

5. Ferrokinetic studies with Fe^{59}, with special emphasis on intravenous plasma iron disappearance rates, red cell iron renewal rate and

percentage of Fe^{59} utilized for erythropoiesis also indicated a normal or increased state of erythropoiesis in these patients.

6. A major factor in the anemia of advanced cancer is an increase in hemolytic activity. In response to this increase in hemolysis the majority of these patients exhibited normal or increased erythropoietic activity which was, however, inadequate to prevent the development of anemia.

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Metastatic Tumors of the Heart*

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THE subject of metastatic tumors of the heart and pericardium has not provoked much interest in the past few years. This is because the incidence was generally considered low and clinical manifestations are the exception.

This report records the relevant findings in a group of autopsies conducted at the Veterans Administration Hospital, Hines, Illinois; in addition a few cases previously hospitalized there and examined at post mortem elsewhere are included. Records and follow-up data have been maintained by the Tumor Research Department of this hospital since 1931 on all hospitalized cases of malignancy.

From 1931 through 1951 more than 21,266 cases of malignant tumor have been treated at this institution. A total of 7,952 autopsies have been performed at this hospital, with the exception of a few elsewhere, and of this number of autopsies 4,124 had malignant disease. Not included in this group are benign tumors, brain and spinal cord tumors and leukemias.

The records of each case of cardiac and pericardial metastases have been reviewed as to pathology, symptomatology and laboratory data. In only a small number was the diagnosis made or suspected before death.

RESULTS

Incidence. The number of cases with heart or pericardial involvement totaled 217, approximately 5.0 per cent. Table I shows the incidence of individual types of tumor. Bronchogenic carcinoma and malignant melanoma show the highest incidence, the remainder are much less frequent. All cases were in males, as the female service at this hospital is very small. The age incidence was thirty to sixty-seven years, the greatest number falling between the ages of thirty-seven and fifty.

The heart was more often involved than the parietal pericardium but both were involved in a goodly number. Table II shows the distribution of these cases.

Spread. It is very difficult to determine the mode of spread accurately unless there is direct extension of the carcinoma from contiguous structures in the area, such as is often seen in bronchogenic carcinoma and occasionally in carcinoma of the esophagus. According to Scott and Garvin,[1] in their 118 cases reviewed at the Cleveland Clinic from 1919 to 1939, true embolic metastasis via coronary arteries was the most common mode of involvement of the heart. In view of the close proximity of the lymphatic trunks to the coronary vessels, according to the work of Rouvière[2] on lymphatic drainage of the heart, we would be inclined to believe that the greatest number of cardiac metastases were

TABLE I

Primary Tumor Metastasizing to Heart	Cases Autopsied (No.)	Heart and Pericardial Metastases	Percentage
Bronchogenic carcinoma	636	80	8.5
Malignant melanoma	76	23	30.3
Sarcoma (not lymph node)	240	14	5.8
Lymphosarcoma (no leukemia)	142	18	13.4
Carcinoma, metastatic, primary unknown	83	10	12.0
Kidney neoplasm (hypernephroma, carcinoma, pelvic carcinoma)	146	7	4.7
Esophageal carcinoma	183	9	4.9
Hodgkin's disease	189	8	4.2
Testicular carcinoma or teratoma	128	7	5.4
Colon carcinoma	144	4	1.6
Pancreatic carcinoma	148	4	2.7
Prostatic carcinoma	194	4	2.1
Adrenal carcinoma	24	3	12.5
Penile carcinoma	33	3	9.1
Gastric carcinoma	408	4	1.0
Parotid carcinoma and mixed tumor	34	3	18.9
Urinary bladder carcinoma	265	3	1.1
Rectal carcinoma	304	2	.7
Lymphoepithelioma	.....	2	....
Lip carcinoma	72	2	2.8
Breast carcinoma (male)	6	1	16.7
Tongue carcinoma	126	2	1.5
Alveolar ridge carcinoma	16	1	12.5
Branchial cyst carcinoma	8	1	12.5
Other carcinomas (respiratory passages, mouth, larynx, anus and thyroid)	424	0	0.0
Total	4,124	217	

* From the Veterans Administration Hospital, Hines, Ill.

due to lymphatic rather than hematogenous spread.

The importance of coincident metastases to the heart or pericardium or both, with involvement of other structures within the bony thorax is indicated in Table III. The primary site of the tumor was within the thorax in 116 cases, outside the thorax in ninety-one, and undetermined in ten instances.

Table IV indicates that involvement of heart and/or pericardium tends to be associated with rather widespread metastases of malignant tumors. In only eighteen instances were the metastases limited to the heart or pericardium.

Pathology. All cases in which the endocardium, myocardium and epicardium (visceral pericardium) were involved have been designated "cardiac" and only those tumors metastasizing to parietal pericardium have been termed "pericardial." Tables VI to IX summarize the gross pathologic findings encountered in our cases. In every case the microscopic features of the metastases corresponded to those of the primary tumor, except for occasional instances of necrosis.

TABLE II

Tumor	Total Cases	Metastases			Total Cardiac	Total Pericardial
		Pericardial Only	Cardiac Only	Both Pericardium and Heart		
Bronchiogenic carcinoma	80	26	21	36	57	62
Malignant melanoma	23	2	13	8	21	10
Sarcoma	14	2	7	5	12	7
Lymphosarcoma	18	2	11	6	17	8
Ca., primary undetermined	10	2	5	3	8	8
Ca. esophagus	9	..	6	3	8	3
Hodgkin's disease	8	1	7	1	8	2
Teratoma	5	1	3	1	4	2
Ca. colon	4	..	2	2	4	2
Ca. pancreas	4	..	3	1	4	1
Hypernephroma	5	..	5	..	5	...
Ca. prostate	4	..	3	1	4	1
Ca. adrenal	3	..	1	2	3	2
Ca. penis	3	..	3	..	3	...
Ca. stomach	4	1	2	1	3	1
Mixed tumor parotid	3	1	2	..	2	1
Ca. bladder	3	..	3	..	3	...
Ca. rectum	2	..	2	..	2	...
Ca. kidney	2	..	2	..	2	...
Ca. pelvis or kidney	2	..	2	..	2	...
Lymphoepithelioma	2	..	2	..	2	1
Ca. testicle	2	..	1	1	2	1
Ca. lip	2	..	1	1	2	1
Ca. breast	1	..	1	..	1	...
Ca. tongue	2	..	...	2	2	2
Ca. alveolar ridge	1	..	1	..	1	...
Ca. branchial cyst	1	1	...	..	...	1
Total	217	39	109	73	182	112

A total of 112 patients had involvement of the pericardium, and of these thirty-nine involved the pericardium alone. (Table II.) The classification in Table V shows the predominant mode of pericardial metastasis, although one type may grade off into the others. Bronchogenic carcinoma involved the pericardium more frequently than other primary tumor in this group, malignant melanoma did so infrequently. In about one-fourth of the cases increased pericardial fluid (Table VI) occurred without parietal pericardial involvement by tumor, and the incidence of bloody fluid was not as high

as might be expected in malignant disease of serous surfaces.

The gross pathologic findings in the cases with cardiac metastases were as variable as in those of the pericardium. Table VII lists the range of heart weight and shows that sixty-five hearts

TABLE III
THORACIC STRUCTURES INVOLVED

	No. of Cases
One or both lungs or pleura	80
Mediastinal structures (mainly lymph node)	57
Both of the above	57
Other structures (ribs, chest wall)	7
No intrathoracic structures except heart	16
Total	217

weighed over 350 gm.; of these, six were considered increased due to other types of heart disease. In many cases the heart weight was not listed, mainly because the heart was bound down with a large intrathoracic mass.

We have classified cardiac metastases according to location and distribution within the

TABLE IV

Metastatic Sites of Tumors Involving Heart and (or) Pericardium in Relation to Number of Organs, Excluding Regional Nodes	No. of Cases
To no organ or region except heart and/or pericardium	18
To only one other organ or region in addition to heart and/or pericardium	30
To two or more other organs or regions and heart and/or pericardium	169
Total	217

heart in Tables VIII and IX. As can be seen diffuse cardiac metastases were approximately as frequent as localized masses. In seventy-two cases of the localized type (a single nodule or group of nodules) the site was specified and in these the left side of the heart predominated. Except when the heart was involved by contiguity the appearance of the tumor was generally nodular and pin-point, rice grain, or up to pea-sized. Usually the nodules were sharply circumscribed but in some cases were conglomerate and occasionally significant necrosis with softening occurred. Tumor nodules were generally situated in the epicardial region, some were in the myocardium, some in both, a few were predominantly in the endocardium. (Table IX.) An epicardial or endocardial tumor may bulge out from the surface, particularly the endocardial tumors which may encroach upon the contiguous heart cavity as a papillomatous propagation of tumor. In ten cases of

TABLE V

Types of Pericardial Metastases, Incidence	No. of Cases
Extension and attachment to mediastinal mass and pericardium alone	25
Extension and attachment to mediastinal mass and pericardium with extension to the heart	25
Pericardium studded diffusely with tumor nodules	12
Pericardium thickened and diffusely infiltrated with tumor	17
Pericardium thickened and diffusely infiltrated with tumor and with extension to epicardium and myocardium*	22
Pericardium infiltrated locally with tumor, without other attachment	11
Total	112

* Bronchogenic carcinoma accounted for twelve instances.

TABLE VI

Pericardial Fluid Encountered	No. of Cases
Simple effusion, over 100 cc.	15
Serofibrinous effusion	5
Purulent effusion*	4
Bloody pericardial fluid, 60 cc. and over†	21
Total	45

* A case of lymphosarcoma.
† Twelve of these cases bronchogenic carcinoma (largest bloody effusion 800 cc.).

predominate endocardial tumor growth, five papillomatous extensions into the heart cavity were noted; one of these formed a dumbbell-shaped tumor which grew from the wall of the right auricle, through the tricuspid valve into the right ventricle. Four of the endocardial tumors appeared to have been implanted directly upon the endocardium; the remaining six appeared to have originated from a myocardial nodule or to have extended directly from a primary bronchogenic carcinoma or mediastinal node. Epicardial metastases frequently showed a tendency to invade the myocardium, this being evident in practically every case microscopically. Involvement of the heart valves, chordae tendinae and septi did not occur frequently. (Table IX.) Tumor cells were not observed within the coronary arteries.

TABLE VII

Heart Weight (gm.)	No. of Cases
350 or under	123
Over 350*	65
Not given	29
Total	217

* Largest weights 850 and 780 gm.

TABLE VIII

Location of Various Cardiac Metastases	No. of Cases
Right auricle	15
Right ventricle*	15
Left auricle	10
Left ventricle†	32
Diffuse tumor nodules or scirrhous type of thickening	78
Localized metastases, not specified	21
Microscopic involvement, no gross disease	6
Tumor thrombus in heart chambers	5
Total	182

* Total in right heart, thirty.
† Total in left heart, forty-two

Of the sixteen cases with intracardiac involvement ten showed invasion of intraventricular septum. Valve leaflets were involved in three, papillomatous extension into the cardiac chambers occurred in five, and invasion of the papillary muscle was noted in two.

Extensive replacement of the myocardium was not frequent, the tumor acting more as a silent invader. Generally, sizable areas of apparently "healthy" myocardium remained, especially with nodular involvement, even when diffuse. Usually the heart was not enlarged or deformed. When this occurred, it was due either to a rapidly growing tumor (as judged both clinically and pathologically) causing a massive, conglomerate, nodular growth or to a scirrhous, markedly fibrosing tumor involving the entire circumference of the pericardium and heart; these situations occurred in thirty-five instances. Malignant melanomas appear to be the most proliferative type of tumor metastasizing to the heart.

TABLE IX

Situation of Cardiac Metastases	No. of Cases
Epicardium predominantly	78
Myocardium predominantly	32
Endocardium predominantly	16
Any combinations of above	35
Not mentioned	21
Total	182

TABLE X

Clinical Manifestations	No. of Cases
Cardiac failure	17
Syncopal attacks	3
Murmurs	3
Pericardial effusion	4
Friction rub (pericardial)	2
Total	29

In addition to tumor, coronary arteriosclerosis was present in thirty instances but usually was mild. Myocardial fibrosis of moderate to marked degree, in addition to tumor, was noted in ten hearts. Other associated cardiac abnormalities were calcified aortic valve with stenosis two, aortic stenosis with insufficiency one, aortic insufficiency three, myocardial infarct two (recent), and unexplained cardiac enlargement one.

Symptoms. In general, symptoms referable

to the heart were absent. Cardiac failure was the most common (Table x), but in one case this was attributable to severe coronary arteriosclerosis with myocardial fibrosis (non-malignant). In only seven of the seventeen cases of congestive failure was this of such degree as to require

configuration in two, pericardial configuration (effusion) in five, displaced heart in one and progressively enlarging heart shadow in two. No reports of chest x-ray were available in a number of cases.

As to analysis of electrocardiograms, only

TABLE XI

Electrocardiogram	No. of Electro-cardio-grams	Site of Metastases						
		Diffuse Nodular	Inter-ven-tricular Septum	Right Heart	Left Heart	Peri-cardial Only	Peri-cardial and Myo-cardial	Other Types of Heart Disease
Normal limits	20	7	4	4	..	2	3	..
Abnormal, non-specific	20	4	1	2	1	5	6	1
Auricular fibrillation	1	1	..	..	..	..	..	..
Anterior coronary occlusion	1	1	..	..	..	..	..	..
Low voltage	6	2	1	1	..	..	1	1
Extra systoles	3	..	..	..	..	2	1	..
Intraventricular block	3	2	..	..	..	..	1	..
Left heart strain	2	..	..	2	1	..	..	1
Right heart strain	2	..	..		..	..	..	..
Pericardial effusion	4	..	..	..	..	2	2	..
Progressive ECG changes	3	1	..	1	..	..	..	1
Total ECG	65	18	6	10	2	11	14	4

intensive therapy. In one of the patients with syncopal attacks, auricular fibrillation at a rapid rate was apparently the cause; the two other cases were apparently due to interference in the conduction mechanism of the heart as there was no evidence of central nervous system metastasis and metastases were present in the interventricular septum and both auricles. A patient with a large tumor thrombus was moribund on admission and died within twenty-four hours. Pericardial effusion was proved by aspiration of bloody fluid. Evidence of tamponade was present in three.

Laboratory Procedures. Routine x-rays of the chest did not aid much in diagnosis. The essential findings were enlarged heart in ten cases, mitral

fifty-seven were taken. The types of electrocardiographic findings in relation to pathological findings can be seen in Table xi. Nonspecific changes predominated. In many of these cases the electrical activity in the heart was not altered despite extensive cardiac involvement by tumor.

Premortem Diagnosis. Seven cases of cardiac and/or pericardial metastasis were diagnosed prior to death. The diagnosis was made rather late in relation to onset of symptoms of malignancy, and in association with widespread malignant disease.

Mode of Death. All records were not complete in regard to the terminal symptomatology. There were seven sudden deaths and one suicide.

The remainder of patients gradually died of cachexia or some other complication of their primary tumor.

COMMENTS

The incidence of metastatic carcinoma of the heart has been variously reported as 0.5 to 19.1 per cent of all malignancies, excluding leukemias. At the time of Yater's article[3] in 1931, metastatic tumor of the heart was considered quite rare. Willis[4] reported an incidence of 6.2 per cent among 323 well studied autposies of tumors; Burke[5] reported fourteen cases in 326 autopsies; Lymburner[6] reported fifty-two cases from 8,550 autopsies of all types; Pollia and Gogol[7] had twenty-nine cases among 1,450 autopsies of malignancies; Herbut and Maisel[9] found forty-five cases in 409 autopsies of malignant tumors. More recent surveys give an incidence of cardiac metastasis of 3.4 per cent by Prichard,[10] while DeLoach and Haynes[11] found an incidence of 12.6 per cent, excluding leukemias. Bisel et al.[12] noted cardiac infiltration in 24 per cent of sixty-nine lymphomas and 12 per cent of 304 other tumors coming to autopsy. Cardiac involvement in leukemia may be as high as 44 per cent[12] in some series. Burnett and Shimkin[13] found an incidence of 14 per cent for metastasis to the heart in 209 tumor cases and an incidence of 29 per cent in leukemia. Young and Goldman[14] found an incidence of 19.1 per cent for such metastasis.

Pritchard[10] has listed certain factors which tend to decrease the incidence of cardiac metastasis: (1) the kneading action of the heart; (2) the metabolic peculiarities of striated muscle; (3) the rapid blood flow through the heart; and (4) the restricted lymphatic connections.

Bronchogenic carcinoma was the most frequently encountered primary tumor in our group and in the series of Scott and Garvin[1] and Herbut and Maisel.[9] Carcinoma of the breast was quite frequently mentioned in all papers but is not included in our study because of its limitations to males. Scott and Garvin's[1] results are also in agreement with ours in showing one-third incidence of bronchogenic carcinoma metastasizing to the cardiac structures. However, Ochsner's report[15] of 3,047 autopsies of bronchogenic carcinoma mentioned that cardiac and pericardial metastases occurred in 12.7 per cent. In other reports the most frequent primary mentioned is kidney, breast and lymph node.[3–6] In all reports sarcoma is a less frequent primary than carcinoma.

Malignant melanoma was frequently encountered in our study, a point stressed in several reports,[13,14,16] and was noteworthy for rapidity of growth in the heart and the marked replacement of functioning cardiac tissue. Practically any tumor can metastasize to the heart and pericardium.[1,3] Thyroid carcinoma has been considered rare, and we have no case; however there are reports of its occurrence.[3,17,18] According to Garvin,[19] Hodgkin's disease rarely implicates the heart. In our study, cardiac but not pericardial metastases were present in a substantial number. In the last few years four cases of Kaposi's disease (angioreticuloendothelioma) involving the heart have been reported.[20,21]

Metastasis to the heart alone is rare. (Table IV.) Willis[4] reports observations of Fergus that bilharzial cancer of the bladder caused a single metastasis to the heart in two cases and observations of Keynes concerning a single nodular metastasis to the myocardium which occurred as the only metastatic deposit of a primary renal carcinoma. Carcinoma of the duodenum[22] and sarcoma of the bladder[23] have been reported in recent years to metastasize solely to the heart.

MODE OF SPREAD

The mode of spread to the heart is frequently speculative but most observers[1,3,4,6,7,9,11] believe that the hematogenous route is the most frequent. Willis[4] has a most interesting chapter on this subject and points out that cardiac metastases are often missed at autopsy due to inadequate sectioning of the myocardium. Harrell[24] quotes a reference on the lymphatic supply of the heart and pericardium that shows many routes available for spread of tumor, particularly from mediastinal metastases. Mediastinal lymph node involvement by malignant disease greatly increases the chances of spread to the heart and pericardium, just as do pulmonary metastases.

The mode of spread to the heart is very difficult to establish unless there is direct extension of the carcinoma from contiguous structures in the area, such as is often seen in bronchogenic carcinoma and carcinoma of the esophagus.

The presence of a subendocardial and subpericardial lymphatic network is now generally agreed upon. The presence of lymphatic vessels or lymphatic capillaries in the myocardium, however, is still disputed. According to Rouvière[2]

the lymphatics of the heart may be classified as follows:

1. The subendocardial network: This network anastomoses with the myocardial lymphatic network. It also diverts its contents into the valvular collecting vessels which transverse the myocardium along the arteries.

2. The myocardial lymphatics: These networks communicate with the endocardial and, on the outer side, with the epicardial networks. In addition, they form in the myocardial substance valvular lymphatic vessels "which flow along and anastomose around the arteries which they accompany to the surface of the heart. Here they empty in the lymphatic collecting trunks of the cardiac sulci." This applies to the lymphatics of the ventricular myocardium. The existence of the lymphatic network in the auricular myocardium has not yet been proved.

3. The subpericardial or epicardial lymphatic network: The existence of this lymphatic network is accepted without dispute. The surface of the ventricles is covered by a rich lymphatic network which is more developed over the apex and the anterior and left lateral surfaces than on the diaphragmatic surface of the heart. This may well account for the large number of cardiac metastases found in the left ventricle. In the lymphatic network of the ventricular epicardium the lymph follows two main currents.

The left region of the epicardial ventricular network extends over the entire left ventricle as well as over that part of the right ventricle which is adjacent to the anterior longitudinal sulcus. The vessels of the anterior ventricular surface empty into two collecting trunks which ascend along the anterior longitudinal sulcus and the left coronary artery. The vessels from the inferolateral surface of the left region unite with two anterior trunks which emerge from the anterior longitudinal sulcus. From this union arises the left principal collecting trunk of the heart. The right region of the epicardial ventricular network covers the entire right ventricle except the portion adjacent to the anterior longitudinal sulcus which is drained by the left collecting trunk. Thus over their entire epicardial route the lymphatic trunks which collect the lymph from both lymphatic regions of the heart travel in company with the coronary vessels and in the fat which surrounds them. In most instances the lymph drained from the left principal collecting trunk is directed, by means of the tracheal bifurcation and the right laterotracheal nodes, to the right jugular subclavian junction. The lymph from the right principal collecting trunk is poured into the left jugular subclavian junction.

As mentioned, the right main stem of lymphatic drainage of the heart empties into the left anterior mediastinal nodes; this group of nodes also receives drainage from the diaphragm, liver, mediastinal pleura, lower thyroid gland and thymus, and passes it on to the right and left jugular subclavian junction. Therefore, tumor cells from one of these organs carried to the anterior mediastinal nodes may enter the cardiac lymphatics by retrograde flow. The left main lymphatic stem of the heart drains into the nodes at the tracheal bifurcation or into the anterior mediastinal node. The tracheal nodes receive drainage from the lower part of the trachea, the bronchi, lungs and esophagus. Carcinoma in any of these organs might metastasize to the heart by retrograde lymphatic spread through the left main lymphatic stem.

It is also well known that when chronic adhesions form between the visceral and parietal pleura, lymphatic vessels grow across and connect the lymphatics of the lungs with these of the chest wall, diaphragm or pericardium. This probably accounts for the large number of cases of bronchogenic carcinoma with extension to the heart.

Recent studies of the direction of the stream as well as the distribution of the valves in the cardiac lymphatics have revealed significant findings. No valves were found in the myocardial lymphatics but they were present in the epicardium. Such valves are more numerous on the anterior surface than the diaphragmatic surface of the heart and are very uncommon in the auricle.

PATHOLOGY

The pathology of cardiac and pericardial metastases is adequately covered by Yater,[3] Willis[4] and Herbut and Maisel.[9] Our findings are in accord with these descriptions. An interesting type of spread from bronchogenic carcinoma which did not occur in our series is that of propagation of the tumor via a pulmonary vein directly into the left auricle, possibly with further extension into the left ventricular cavity.[25–27]

There has been some division of opinion as to which side of the heart tumors are more likely to metastasize, the right,[3,10] left[1,14] or diffusely.[9] Probably the reason for this variation is the types of primary tumor most often encountered

in a particular series. In this series, in which bronchogenic carcinoma was most frequent and tumor spread more often hematogenous via pulmonary veins, it is not surprising that left heart involvement was more common.

CLINICAL MANIFESTATIONS

Generally, no clinical findings are present unless cardiac metastases are extensive. Cardiac failure or conduction disturbances (usually detected in the electrocardiogram) are the most common clinical manifestations. It is apparent that metastatic tumor to the heart or pericardium may give rise to symptoms as the result of one or more of the following conditions: (1) invasion of the myocardium with replacement, leading to congestive failure; (2) interference with coronary flow, leading to angina pectoris or congestive failure and possibly infarction; (3) distortion of the cardiac valves; (4) intracardiac obstruction by new growth; (5) disturbance of rhythm or conduction; (6) compression of the heart by fluid or diffuse invasion of the pericardium; (7) rupture of the myocardium; (8) embolism originating in tumor thrombi. It is remarkable that so few clinical manifestations develop, even with severe destruction and replacement of cardiac muscle. Lisa and others[28] point out other findings that do occasionally occur, namely, valvular disease resembling mitral stenosis, anginal pain and Stokes-Adams syndrome. According to these authors there are no diagnostic signs or symptoms; however, the onset of cardiac dysfunction in the presence of widespread malignancy or intractable cardiac failure of obscure cause should make one suspect the presence of tumor in the heart.

Pericardial metastases are usually silent, more so than myocardial tumors, and pericardial effusion is the significant clinical finding.[7,29] Aspiration should be performed and the sediment studied for tumor cells. Hemorrhagic fluid is significant but was present in only twenty-one of forty-five patients in this series. Constrictive pericarditis may result from pericardial metastases.[30,31]

Electrocardiograms may be normal with considerable myocardial involvement and only occasionally reveal diagnostically helpful information, according to our experience. This is borne out by Siegel and Young[32] who state there is no uniformity of findings; their most common abnormality was a variable inversion of T waves and low voltage. Coronary infarction patterns have been reported,[32,33] also disturbances in conduction and arrythmias.[35,36]

X-ray of the chest has rarely been helpful in diagnosis in our series. In reviewing the literature, the x-ray findings were rarely of diagnostic significance. Pericardial effusion and cardiac enlargement are the most important findings.

Doane and Pressman[37] summarized all cases of metastatic malignancy of the heart to be diagnosed prior to death; there were twenty cases to that date. In 1947 Brick and Greenfield,[38] in a survey to that date, found twenty-two instances of antemortem diagnosis of tumor of the heart. Four of these were primary tumors. To this series they added one case of reticulum cell sarcoma diagnosed before death. Later, instances of antemortem diagnosis of metastasis from carcinoma of the cecum[39] and malignant melanoma were added.[40] Dimmette[41] in 1950 reported four instances in which the antemortem presumptive diagnosis of metastasic lesions of the heart was made. DeLoach and Haynes[11] added two more such instances, as did Burnett and Shimkin.[13] The antemortem diagnosis was made seven times in this series of cases.

Death usually is referable to complications of tumor other than the metastases to the heart, namely, cachexia, hemorrhage, infection and the like. This was noted in our group and in articles mentioned. In the past, sudden death has been regarded as fairly frequent but this was not apparent in our cases. There are occasional cases with sudden death due to Stokes-Adams syndrome and ball-valve action of obstructing tumor thrombi. We have not met with acute embolic phenomenon of tumor masses from the heart. One patient was reported to have died of cardiac rupture.[22]

SUMMARY

An analysis of the findings in 217 cases of neoplasm with cardiac and pericardial metastases is presented. The clinical and autopsy data are correlated.

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Association of Gastrointestinal Carcinoid Tumor with Cardiovascular Abnormalities*

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A SYNDROME of malignant intestinal carcinoid with hepatic metastases and fibrostenotic lesions predominantly of the pulmonic and tricuspid valves has recently been described. Isler and Hedinger[1] reported three of their own cases and commented on another. The three cases were individuals in the fourth, fifth and sixth decades. All had clinical evidence of right-sided heart failure with dyspnea, cyanosis, neck vein distention, peripheral edema and cardiac murmurs. At postmortem examination carcinoid tumors of the small intestines were present. These had metastasized extensively to the liver. In two instances there were fibrostenotic changes in the pulmonic and tricuspid valves together with a more diffuse endocardial fibrosis. In the third case the valvular lesion was described as a form of "chronic recurrent verrucous endocarditis." In one case there was also some involvement of the mitral valve. Rambo, Gore and Vance[2] described another case with essentially the same clinical and autopsy findings. They postulated that the valvular alterations might perhaps have developed secondarily to some biochemical effect on the endocardium produced by a substance liberated by the carcinoid tumor tissue.

In order further to evaluate the incidence and pathogenesis of this syndrome twenty-six autopsied cases of carcinoid tumors of the gastrointestinal tract, derived from an autopsy series of 8,000 adults, were analyzed. In reviewing these cases particular attention was directed to any developmental or acquired cardiovascular defects, the primary site and size of the carcinoid tumors, and the extent and distribution of the metastases. In addition, 500 autopsies sampled at random from the entire series of 8,000 were also studied relative to the presence of developmental cardiovascular defects and fibrostenotic lesions limited to the pulmonic and tricuspid valves. In both groups of cases, those with carcinoid and those without, such findings as probe patency of the foramen ovale, fenestrations of the semilunar valves and minor accessory coronary orifices were ignored. The incidence of these alterations was the same in both groups and was in keeping with well established figures.[3]

The observations concerning carcinoid tumors, metastases and cardiovascular abnormalities are presented in Table I.

In the 500 autopsies on adults without carcinoid tumors, less than fifty (10 per cent) showed any developmental defects of the heart (excluding the abnormalities mentioned previously). In contrast to this, in the twenty-six cases with carcinoid tumors there were nine (33 per cent) with unequivocal congenital defects or anomalies. In the control group there was not a single instance of isolated fibrostenotic involvement of the tricuspid and pulmonic valves. These valves were involved only in cases of rheumatic heart disease, in conjunction with involvement of the valves on the left side of the heart. In the cases with carcinoid tumors there were ten with varying degrees of right-sided valvular lesions; in none of these were there any stigmata of rheumatic heart disease. All told, eighteen of the twenty-six cases exhibited some cardiovascular abnormality or defect.

Among the defects were two hearts with true Chiari's net. The reported usual incidence of this finding is once in 500. Dextrocardia with an atretic right auricle and a pulmonic valve with four cusps are not very common anomalies yet these were present in this small group of cases. The incidence of the isolated tricuspid and pulmonic valvular changes appears even more significant.

Among the twenty-four cases in which the carcinoid was primary in the intestinal tract

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TABLE I

Autopsy No.	Age and Sex	Primary Site of Carcinoid	Sites of Metastases	Cardiovascular Abnormalities
9745	41, M	Ileum	Liver, mesentery, lymph nodes	Hyaline nodules on anterior pulmonic valve cusp
10101	55, M	Ileum	None	Dextrocardia; thin-walled right auricle
10972	61, M	Ileum	Liver, lungs, pleura, lymph nodes, thyroid	Edematous thickening of tricuspid valve; subpulmonic valve; endocardial fibrosis
11651	66, M	Ileum	Liver, heart, lymph nodes	Marked sclerosis of pulmonic and tricuspid valves; hypertrophy of right ventricle; aortic arch malformation
11877	72, M	Ileum	Lungs	Endocardial fibrosis, right ventricle; extensive scar below pulmonic valve
12443	47, F	Ileum	Liver, lungs, spleen	Marked sclerosis of pulmonic valve with distortion; moderate fibrosis of tricuspid valve
13025	65, F	Ileum	None	Chiari's network; coronary sinus valve
13070	42, F	Rectum	None	Anomalous aortic arch
13237	43, M	Rectum	None	None
13599	46, M	Ileum	Liver, lymph nodes	Tricuspid and pulmonic valve sclerosis, marked; anomalous aortic arch
13733	64, M	Ileum	None	None
13827	70, M	Stomach	None	Chiari's network; moderate fibrotic thickening of tricuspid valve
14480	63, M	Ileum	Lymph nodes, ovary	None
14695	46, F	Ileum	Liver, pleura, lymph nodes	Widely patent foramen ovale
14880	55, M	Ileum	None	Widely patent foramen ovale
14934	42, F	Ileum	None	None
14961	70, M	Ileum	None	Separate left circumflex artery arising from aorta
14966	68, F	Ileum	Lymph nodes	None
15243	38, M	Cecum	Liver, lungs	None
15313	81, M	Ileum (multiple)	None	Four pulmonic cusps
15322	54, M	Ileum	None	None
16572	60, F	Ileum	None	None
17049	64, M	Ileum	None	Focal scarring of endocardium, both ventricles; sclerosis of mitral valve
9839	66, M	Ileum	None	Fibrotic thickening of tricuspid valve with roughening of edge of infundibular leaflet
11386	44, F	Appendix	None	Edematous thickening of tricuspid valve; widely patent foramen ovale
11495	64, M	Appendix	None	None

(excluding the two appendiceal cases) were ten with metastases. This finding is in general agreement with the experience of others.[4] There was a notable difference, in the distribution of the cardiovascular lesions, between those with and those without metastases. Seven of the ten cases with metastases demonstrated endocardial fibrosis with involvement of the pulmonic and tricuspid valves. Only three of the sixteen without metastases had any of these alterations. The other defects were more common in the cases without metastases.

The endocardial changes varied in age, severity and extent. In some there was minimal fibrotic thickening which was diffuse or focal; in others it was extensive and resulted in marked anatomic distortion of the valves. In some instances the valves exhibited an earlier and edematous type of involvement. In one case a fresh non-bacterial thrombotic or verrucous type of endocarditis was superimposed upon a scarred valve. In a few instances there was fibrosis, at times extensive, in the endocardium beneath the pulmonic valve ring. In one case mitral valve involvement also was present. The older and more advanced changes were found in those cases in which the carcinoid had metastasized; however, advanced changes were not necessarily limited to those with extensive hepatic metastases.

DISCUSSION

It is quite clear that the syndrome is not merely a coincidence of conditions. There is no ready explanation for the differences in the cardiovascular findings in those cases with metastases and those without. However, because of the high incidence of developmental cardiovascular alterations in the group as a whole, and because there is justification for regarding carcinoid tumors as being developmental in origin, it is possible that there is a basic defect in the valvular substance which during life may progress to marked valvular damage.

Rambo, Gore and Vance advanced the concept that the progressive and acquired changes in the valves may be related to some substance derived from the tumor tissue which exerts a deleterious effect on the endocardium. The discharge of this material into the right side of the heart, particularly if derived from liver metastases, might account for the predominance of the changes in this area. The carcinoid tumor is thought to originate from the Kulschitzky cells which by some are claimed to have a neuroendocrine function.[5] Pernow and Waldenstrom[6] have recently demonstrated the presence of substances resembling 5-hydroxytryptamine (serotonin) during attacks of flushing in the blood and urine of two patients with carcinoid tumor. They also indicate that there is sufficient evidence now to be certain that this substance, or substances similar to it, are secreted by the intestinal argentaffin cells. They also quote a report by Lembeck who has recently found that benign carcinoid tumors contain large amounts of 5-hydroxytryptamine. This would support the concept of Rambo, Gore and Vance. The progression of the lesion on the valves may also be dependent on recurrent episodes of a non-bacterial thrombo-endocarditis with subsequent scarring. Such a lesion was observed in one of the cases in this group and another was reported by Isler and Hedinger.

Other instances of the association of developmental neoplasms with non-neoplastic anomalies are known. One such example would be the association of congenital rhabdomyoma of the heart and renal cysts with tuberous sclerosis. Evidence for the developmental origin of carcinoid tumors is found in the early age at which they are observed in the appendix; their frequent and characteristic location near the tip of the appendix; the multiplicity of primary sites in individual cases; and the peculiar type and rate of growth.

Since patients with carcinoid may survive for many years even with extensive metastases there is time for the valvular lesions to develop to the extent that they may be recognized clinically. Perhaps biochemical study of these cases, as suggested by others, may provide an explanation for the progression of the basic developmental cardiovascular defect.

It should be noted that the valvular changes, at least in their end stages, are anatomically indistinguishable from those that may occur in rheumatic heart disease.

SUMMARY

1. Carcinoid tumors of the gastrointestinal tract appear to be associated with a high incidence of cardiovascular abnormalities.

2. In addition to cardiovascular developmental defects or anomalies, acquired endocardial lesions predominantly involving the tricuspid and pulmonic valves are frequently present.

3. The most advanced involvement of the tricuspid and pulmonic valves is found in those cases in which the carcinoid tumor has metastasized.

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Dermatomyositis: Diagnostic Features and Therapeutic Pitfalls*

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IT is the purpose of this report to describe two cases which (1) highlight the value of urinary creatine determinations and muscle biopsy in arriving at a diagnosis of dermatomyositis, (2) conversely, delineate the clinical features of dermatomyositis which make possible a positive diagnosis in the face of incomplete laboratory confirmation, and (3) illustrate hazards which beset ACTH-cortisone therapy in this disease.

Dermatomyositis, the rarest of the so-called collagen diseases,[1] was first described in 1863 by Wagner.[2] Scheuermann[3] was able to review 263 cases in 1939 and the number of reported cases is now in the vicinity of 600.

Dermatomyositis is difficult to define with precision because it is primarily a clinical entity, with little pathologic or biochemical distinctiveness. In 1922 Steiner[4] described it as "an acute, subacute or chronic disease of unknown origin characterized by a gradual onset, with vague or indefinite prodromes, followed by edema, dermatitis and a multiple muscle inflammation." This was modified in 1935 by Sydenstricker and Thomas[5] to read "non-suppurative muscle inflammation."

It is now possible to describe dermatomyositis more exactly as a collagen disease in which non-suppurative polymyositis with creatinuria is the distinguishing characteristic, commonly accompanied by dermatitis and edema. Emphasis on creatinuria in dermatomyositis is justified because in no other collagen disease is this abnormality so striking and so consistent.

CASE REPORTS

CASE 1. A fifty year old retired Master Sergeant first sought medical aid at the Reno Veterans Administration Hospital, July 11, 1953, because of pain in the right shoulder. The shoulder area was injected with procaine for relief of "bursitis." The next day dyspnea and right chest pain developed which proved to be due to pneumothorax. The patient was hospitalized for thirty days and the lung expanded satisfactorily. Studies for tubercle bacilli were negative.

After leaving the hospital he was asymptomatic until August 22, 1953, when pain in the right shoulder recurred, followed in the next two weeks by migratory polyarthritis involving the shoulders, hands, knees and back. Intermittent swelling without redness was present in the peripheral joints. Five days prior to admission a cough productive of thick white sputum developed.

On admission to the hospital, September 10, 1953, the patient was almost immobilized by severe articular pain. Physical examination showed swelling of the wrists and signs of pleural fluid in the right chest. Initial laboratory studies showed normal urine, leukocyte count, hemoglobin and sedimentation rate (4 mm./hr., Wintrobe). Chest roentgenograms showed fluid covering the lower one-third of the right lung field and a hazy infiltrate in each apex. (Fig. 1.) He was able to produce only one scanty twenty-four-hour sputum sample; this and three gastric washings were negative for tubercle bacilli. Second strength tuberculin (PPD) was positive.

His cough and expectoration cleared up within a week without therapy. Within four weeks the pleural reaction subsided with minimal residual thickening, and the apical densities disappeared. In the meantime arthritis became the dominant feature of his illness. Joint pain became less migratory and more persistent, and periarticular thickening about the shoulders, wrists,

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metacarpophalangeals and knees progressed at an alarming rate. About the tenth hospital day it was noted that weakness of the distal muscles of the upper extremities was developing, quite out of proportion to the severity and duration of his arthritis. The muscle bellies were not swollen or tender but atrophy was evident. Effusion appeared in the right knee and moderate edema of the ankles developed. Pale amber fluid, 20 cc., was aspirated from the right knee and showed 2,800 leukocytes per cu. mm. of which 1 per cent were eosinophils. The serum uric acid was 2.3 mg. per cent, the serum NPN 35 mg. per cent, the serum albumin was 2.7 gm. per cent and the serum globulin 3.7 gm. per cent. The sedimentation rate rose to 52 mm. per hour. A serum test for L.E. cells was negative.

A tentative diagnosis of dermatomyositis was made.

Urinary excretion of creatine was found to be 780 mg. per twenty-four hours. (Table 1.) Biopsy of the right gastrocnemius muscle was reported to show: "several fragments of skeletal muscle fibers; in several areas there are degenerative changes. The interstitial vessels show a diffuse chronic inflammatory infiltrate in perivascular regions. This infiltrate consists of lymphocytes and monocytes. In an occasional area inflammatory cells are noted in the wall and within the lumen of the vessels. A larger area reveals a diffuse chronic inflammatory process between fragments of eosinophilic amorphous staining material, suggesting degenerative skeletal muscle fibers and collagen bundles. This process is similar to those around the vessels, but in addition there are plasma cells and cells with pale nuclei suggesting reticulum cells. The vessels in this area show marked degeneration of their walls, with eosinophilic hyaline material within the lumen. There is an absence of granulation tissue, neutrophils and eosinophils. Diagnosis: Dermatomyositis, gastrocnemius muscle, right."

By the fourth week in the hospital fibrous ankylosis of the involved joints was detectable despite the use of salicylates and physiotherapy. Hydrocortone, 20 mg. q.i.d., was started orally on the thirty-fourth hospital day, with prompt relief of pain and stiffness. However, periarticular thickening and limitation of motion were only slightly ameliorated. The erythrocyte sedimentation rate fell to 11 mm. per hour, and the serum albumin was 3.3 gm., globulin 3.1 gm. per 100 ml. As can be seen from Table 1, a gratifying drop in urinary excretion of creatine occurred. To our chagrin, however, this change toward normal occurred three days *before* hydrocortone was started.

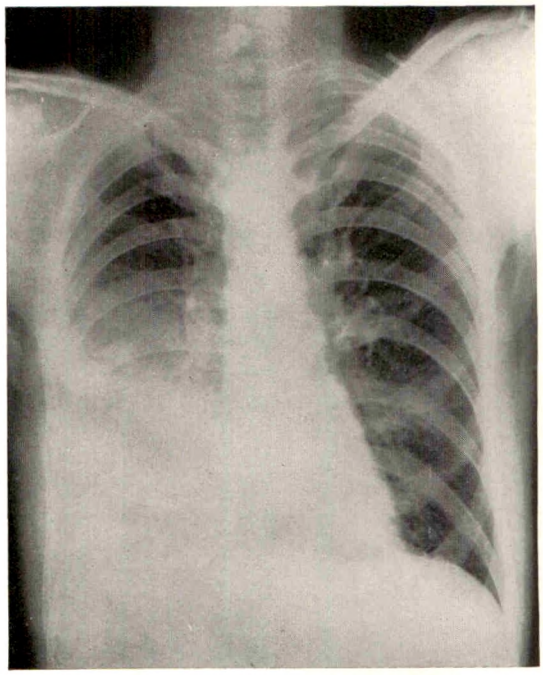


FIG. 1. Case 1. Right pleural effusion and bilateral apical infiltrations present at the time of first admission to hospital.

Although he had been bedridden before beginning treatment, the patient became hyperactive after starting hydrocortone. He had insomnia and was alternately euphoric and bellicose. At his insistence he was released from the hospital on the nineteenth day of hydrocortone therapy.

Outpatient follow-up November 17, 1953, showed continued improvement. On December 1, 1953, cortisone, 67.5 mg. per day, was substituted for hydrocortone and gradual withdrawal of the hormone was begun. On December 15 he complained that pain and stiffness in his wrists and knees were accentuated. The effusion in his left knee had recurred and fibrous ankylosis of his shoulders had progressed to the point where abduction was limited to about 25 degrees to each side. He complained of "heartburn" and an episode of nocturnal vomiting. He vigorously denied ever having had any gastrointestinal symptoms prior to his present illness. Because of the danger of a cortisone-induced peptic ulcer, antacid medication was started and the dose of cortisone was further reduced to 50 mg. per day.

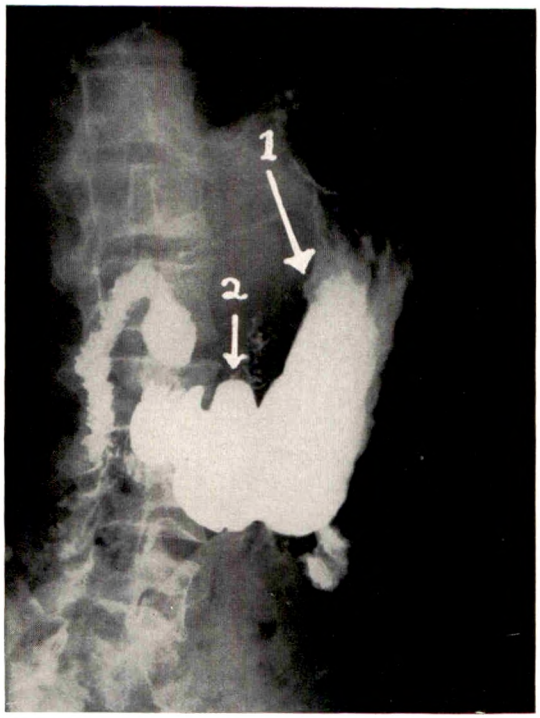


FIG. 2. Case 1. Small ulcer (1) high on lesser curvature of stomach, and (2) large prepyloric ulcer which simulates a peristaltic wave but on fluoroscopy was fixed and failed to transmit peristalsis.

His epigastric distress worsened and on December 28, 1953, he vomited approximately one quart of coffee-ground material and passed two tarry stools. He was admitted to the hospital in shock, which responded to transfusions. An upper gastrointestinal series three days later showed a large ulcer crater on the pyloric portion of the lesser curvature of the stomach. A second ulcer, considerably smaller, was later demonstrated higher on the lesser curvature. (Fig. 2.) The patient left the hospital against advice on the eighth day. He adhered to an ulcer regimen and roentgenograms of the stomach showed progressive healing of both gastric ulcers, with complete disappearance by March 10, 1954.

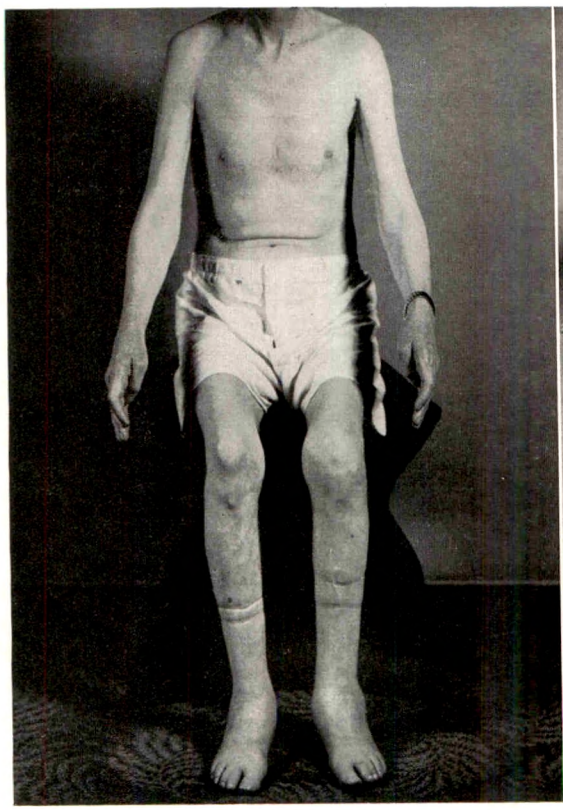
Cortisone was stopped at the time of his hematemesis, and his stiffness progressed: motion of his mandible was restricted to approximately 15 degrees, and he could no longer close his fingers into a fist. Atrophy of the calf, shoulder and interosseous muscles of the hands was pronounced. Fasciculation of the muscles of his upper back was noted. His gait was slowed to a shuffle by pain across the dorsa of his feet and persistent edema of his feet and ankles. Excretion of creatine dropped briefly to normal (February) after discontinuation of cortisone but then resumed the level which had been present before steroid therapy. (Table 1.) Mixed tocopherols (50 mg. twice daily) and testosterone propionate (25 mg. intramuscularly twice weekly) were administered for the next three months, during which time slow but unmistakable improvement in muscular strength and joint mobility occurred. Figures 3 and 4 illustrate the muscular atrophy, edema and articular changes present at this time.

TABLE 1
TWENTY-FOUR HOUR CREATINE* AND CREATININE EXCRETION (CASE 1)

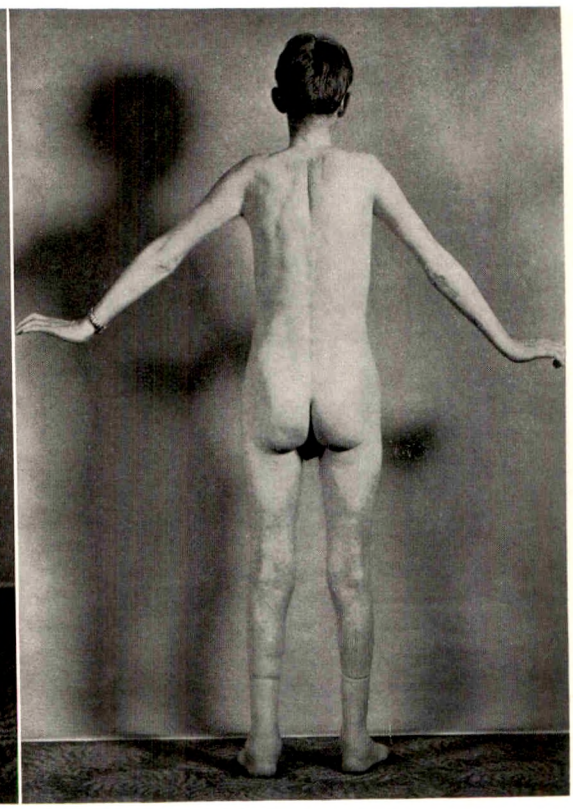
Date	Creatine* (mg./24 hr.)	Creatinine (gm./24 hr.)	Urine Volume (cc./24 hr.)
Sept. 21, 1953	780	2.5	2600
Oct. 12, 1953	600	2.65	2805
Oct. 19, 1953	222	2.1	1800
Oct. 28, 1953	272	1.7	1800
Nov. 3, 1953	238	1.76	1925
Nov. 8, 1953	232	1.6	1980
Nov. 19, 1953	134	1.2	1875
Dec. 28, 1953	267	1.7	2230
Feb. 10, 1954	85	1.7	1200
Mar. 10, 1954	464	2.2	1750
Apr. 27, 1954	209	1.5	1480

* Method of creatine determination. Hawk, P. B., Oser, B. L. and Summerson, W. H. In: Practical Physiological Chemistry, 12th ed., p. 509. Philadelphia, 1937, P. Blakiston's Son & Co. The normal range is 0–200 mg./diem.

In this case a collagen disease was first suggested by the coexistence of pleural effusion and arthritis. A negative L.E. cell test ruled out systemic lupus erythematosus, and the rapid development of persistent joint damage excluded rheumatic fever. The arthropathy was typical of rheumatoid arthritis but this was not considered to be the definitive diagnosis because: (1) muscular weakness and atrophy developed with unusual rapidity and severity, and (2) the disease began with pleuropulmonary involvement. Visceral lesions may occur in rheumatoid arthritis but usually late in the disease and they are often subclinical, being discovered at autopsy. A great variety of serious visceral manifestations has recently been reported in patients with rheumatoid arthritis, including pleuritis, pneumonitis, pericarditis, pathologic



3



4

FIG. 3. Case I. Involvement of wrists, hands, ankles and left knee. Edema of legs.

FIG. 4. Case I. Mild to moderate atrophy of muscles of legs, upper back and shoulder girdles. Shoulders and wrists are maximally extended, showing degree of ankylosis ten months after onset of illness.

findings of periarteritis nodosa[6] and false positive serum L.E. tests,[7] following treatment with cortisone. The pleuropulmonary lesions in our patient appeared concomitantly with the joint symptoms and before cortisone was administered.

Confirmation of the diagnosis of dermatomyositis was afforded by the marked creatinuria and by muscle biopsy. This patient's persistent failure to develop dermatitis is unusual but similar cases of dermatomyositis have been reported.[8]

The two gastric ulcers in this patient were probably secondary to steroid therapy. The fact that he had had no gastrointestinal symptoms prior to ingesting hydrocortone and cortisone, and the healing which followed withdrawal of the hormones support this conclusion. The possibility remains that the ulcers resulted from dermatomyositic involvement of the submucosal blood vessels with infarction of the gastric mucosa. Gastrointestinal ulceration on this basis has been demonstrated at autopsy in dermatomyositis.[9]

CASE II. A twenty-two year old housewife was admitted to the hospital September 22, 1953, complaining of a skin rash. The eruption began on August 15th with a few small papules on the left wrist. Three days later penicillin, 300,000 units t.i.d., was given at another hospital in conjunction with a minor operation on the urethra. The following day an eruption on her breasts developed which within a week spread over her entire body, sparing only the face and back. On admission she presented many large scaling plaques (3 to 8 inches in diameter) which were dry and intensely pruritic. The plaques had raised edges and no central clearing. The scale was white, hard and adherent. There was no ulceration or mucous membrane involvement. All lymph nodes accessible to palpation were moderately enlarged and slightly tender.

Past history revealed no previous allergic phenomena and was otherwise non-contributory.

Initial laboratory studies, including urinalysis, complete blood count, serologic tests and chest roentgenogram were normal. A consulting dermatologist reported a punch biopsy of the skin as "negative," and suggested a tentative diagnosis of penicillin reaction. Neurodermatitis was considered because of the fact that one month before her rash appeared the patient had witnessed the death of one of her children in an automobile accident.

Response to antihistaminics and topical therapy was poor, and she was given ACTH by intravenous drip for four days and then intramuscularly for eight days. There was doubtful improvement and therapy was changed to cortisone for one week in decreasing dosage. Her temperature reached 100°F. on three occasions. At the time of dismissal on the twenty-second day there was slight improvement in her skin. She remained at home only five days. Although her dermatitis became much worse, her readmission was actually prompted by muscular rather than cutaneous symptoms. During her first hospital stay she had complained of a great deal of back pain, and the paraspinal muscles were remarkable tender to palpation. At that time the significance of this observation escaped us. After she left the hospital similar pain appeared in her arms and legs. All of the involved muscles developed an inordinate degree of weakness in a short time and on readmission to the hospital the patient was unable to walk because of pain and weakness in her legs.

Examination showed that she was acutely and severely ill, with a temperature of 103.6°F. Her body was covered from scalp to toes with a dry exfoliation, severely pruritic, and the underlying skin in a few areas showed purplish mottling suggestive of small ecchymoses. Mild edema, both subcutaneous and intracutaneous was present over the entire body, most pronounced periorbitally. The muscles of her back and extremities were exquisitely tender to pressure. The lymphadenopathy previously noted was accentuated. There was tachycardia (120/min.) and a systolic murmur grade II in the pulmonic area.

Urinalysis and complete blood count were again normal, without eosinophilia or lymphocytosis. Fluoroscopy of the chest showed that the cardiac silhouette was somewhat more globular than before, and the amplitude of cardiac pulsations was diminished, suggesting pericarditis. An electrocardiogram showed right axis deviation, low amplitude QRS in Lead I, aVR and aVF, T waves upright in Lead I, inverted in Lead III and flat in aVF. The transition zone appeared in lead V_5. A second electrocardiogram seven weeks later was normal.

TABLE II
TWENTY-FOUR HOUR CREATINE AND CREATININE EXCRETION (CASE II)

Date	Creatine (mg./24 hr.)	Creatinine (gm./24 hr.)	Urine Volume (cc./24 hr.)
Oct. 22, 1953	465	2.0	1600
Nov. 2, 1953	279	1.26	1860
Nov. 4, 1953	162	1.1	1250
Nov. 17, 1953	1680	1.73	2650
July 2, 1954	168	1.6	1400

A tentative diagnosis of dermatomyositis was made.

Urinary excretion of creatine the day after her second admission was 465 mg. in twenty-four hours. (Table II.) Biopsy of the skin and right gastrocnemius muscle three days after admission was reported to show "intact muscle fibers separated by loose mildly vascular fibrous connective tissue. A moderately thickened, parakeratotic epidermis which exhibits mildly elongated club-shaped rete pegs, a thin granulosal layer, and frequent cells showing intracellular edema. A few small aggregations of segmented neutrophiles are present within the parakeratotic lamina. The vascular dermal papillae are elongated, extend close to the surface, and contain scattered lymphocytes, occasional segmented neutrophiles, and a few melanin laden macrophages. The underlying dermis exhibits many capillaries cuffed by lymphocytes. Diagnosis: Skeletal muscle, no diagnostic features; dermatitis, chronic, nonspecific."

ACTH was reinstituted October 23rd immediately after the biopsy. Within four days fever subsided and she was able to eat and sit up in bed. Exfoliation continued however at a brisk pace. Raw fissures appeared in the flexural creases under the breasts, at the elbows and groins. She complained bitterly of a feeling of tightness in her skin and refused to move for fear her skin might crack open. The itching and dryness could be controlled only by keeping her swathed from head to toe in lanolin dressings. Despite all efforts she shed great quantities of skin and lost weight steadily.

A second dermatologic consultation was obtained, and a third biopsy was taken from the right deltoid area. This was reported to show features "highly suggestive of psoriasis, which can occasionally exfoliate." Urine analysis for arsenic was negative. A serum test for L.E. cells was negative on two occasions. The serum protein-bound iodine was 6.6 μg. per 100 cc., NPN 28 mg. per cent, creatinine 1.0 mg. per cent, total cholesterol 283 mg. per cent, and cholesterol esters 206 mg. per cent. The serum contained 3.7 gm. of globulin and 3.3 gm. of albumin per 100 cc.

Withdrawal of ACTH was begun. Sutures sloughed from both biopsy wounds and healing was slow. By the twentieth day her lymphadenopathy and muscle tenderness were gone and she was able to walk. However, muscular weakness was extreme, and syncope due to postural hypotension occurred on several occasions. During the fourth week in the hospital painful erythematous stomatitis developed which cleared with local measures.

On the twenty-seventh day creatine excretion reached the remarkable level of 1,680 mg. in twenty-four hours. Review of the second biopsy by the Armed Forces Institute of Pathology was reported to show: "changes in the epidermis which tend to be psoriasiform but are not diagnostic of this entity. The changes in the muscle are those of an increased number of sarcolemma nuclei and slight degeneration of the muscle fiber."

Bilateral acute otitis media with fever (103°F.) developed on the thirtieth day. This responded to terramycin.® About the thirty-fifth day striking subsidence of the exfoliation was noted. Despite the encouraging progress of her skin lesions the patient remained depressed. Throughout her illness she had been rather mute and required much reassurance and cajoling to get her to eat, to resume walking and to try to help herself. On the thirty-ninth day she abruptly became violently agitated, with delusions and paranoid traits. She was transferred to a Neuropsychiatric Hospital.

She returned to us for follow-up examination seven months later, nine months after the onset of her illness. Her psychosis had cleared after withdrawal of ACTH and a long course of electroshock and insulin shock therapy. All of her muscular pain, tenderness and weakness were gone, she had regained her normal weight and felt entirely well. A complete blood count and urinalysis were normal. Urinary excretion of creatine was 168 mg. in twenty-four hours.

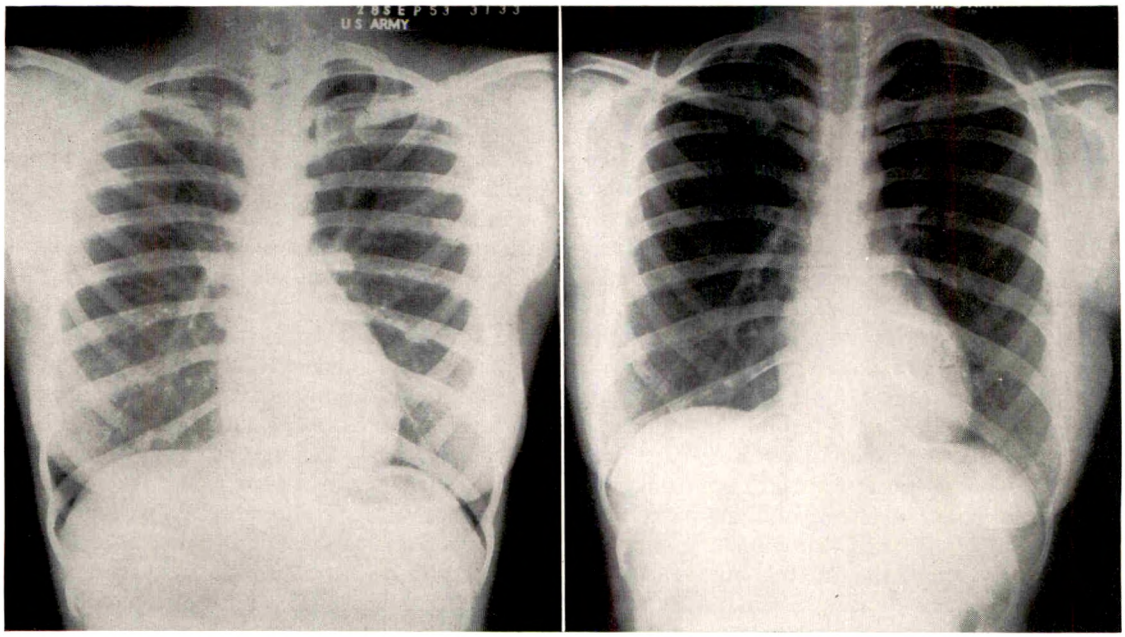
Although myositis and creatinuria are no longer in evidence at the time of writing, her present state probably represents remission rather than recovery since she still has moderate exfoliation involving the hands and feet, and serial chest roentgenograms show an increase of 1.5 cm. in the transverse diameter of the heart. (Figs. 5 and 6.)

The classic triad of polymyositis, edema and dermatitis was so striking in this patient that a firm diagnosis of dermatomyositis was possible in the face of negative biopsy reports. The intense creatinuria did much to bolster the diagnosis. Eventually a biopsy report consistent with the diagnosis of dermatomyositis was obtained.

Despite the fact that the microscopic changes described were consistent with dermatomyositis, this diagnosis was not ventured by the pathologists who reviewed the biopsies. This underlines an important point: the microscopic findings in dermatomyositis are non-specific and occur in several diseases. At best, when the microscopic changes are well advanced, as they were in the biopsy from our first patient, a pathologic diagnosis of dermatomyositis may be advanced, but not without hesitation. Furthermore, the chances of sampling a histologically involved site even in unquestionable dermatomyositis are far from 100 per cent. In short, a positive biopsy however desirable is not a *sine qua non*.

Psoriatic dermatitis[10] and exfoliative dermatitis[2] may occur in dermatomyositis but the combination of both in a single patient has not previously been reported. Stomatitis of the type presented by this patient occurs in approximately one-third of patients with dermatomyositis.[3,11] The spontaneous disruption and delayed healing of both biopsy wounds was disconcerting, and may have been related to ACTH therapy. Wound disruption (laparotomy) was noted by Cottell[12] in a patient with dermatomyositis who was not under treatment with ACTH.

Electrocardiograms and fluoroscopy gave evidence of cardiac involvement but were not specific enough to permit a definite diagnosis of either pericarditis or myocarditis. Enlargement of the cardiac silhouette on follow-up chest roentgenogram nine months after onset of her illness was additional evidence of cardiac involvement. Electrocardiographic abnormalities are frequent in dermatomyositis but pericarditis is rare.



5 6

FIG. 5. Case II. Chest roentgenogram, first month of illness. Normal cardiac silhouette.

FIG. 6. Case II. Ten months after onset. Increase of 1.5 cm. in transverse diameter of heart. Multiple films demonstrated persistence of the enlargement in all phases of respiration and during the Valsalva maneuver. Normal cardiac pulsations on fluoroscopy and a normal electrocardiogram suggest that this represents the effects of myocardial rather than pericardial involvement.

As with our first patient, a major complication necessitated the discontinuation of steroid therapy. Although her psychosis may have been precipitated by ACTH, it may actually have been an expression of dermatomyositic cerebral involvement since distinct pathologic lesions, involving particularly the frontal lobes, have been described in patients with dermatomyositis.[13]

REVIEW OF THE LITERATURE

Because our cases presented many unusual features it seemed desirable to determine the outer limits of the syndrome of dermatomyositis. It is evident from a review of the literature that while the fundamentals of dermatomyositis are polymyositis with creatinuria, dermatitis and edema, this disease is as protean in its manifestations as any other collagen disease.

Dermatitis. The dermatitis in dermatomyositis varies almost beyond belief. It may be erythematous, erysipeloid,[14] macular, papular,[4] telangiectatic,[15] purpuric, vesicular, bullous,[16] ulcerative, exfoliative[2,17] or psoriatic.[10,18] The skin may be shiny or dull, thickened or atrophic,[19] scaly or like wrinkled parchment. The accessory skin structures are often involved: hypertrichosis[19,20] and alopecia,[8,19,21,22] either patchy or diffuse, have been described. Hyperhidrosis,[22] photosensitivity[13] and Raynaud's phenomenon[11,21] may occur. Pigmentation, localized or generalized, may occur alone or with areas of depigmentation in the same patient.[13,19] Follicular keratosis[23] and pitting of the fingernails[18] have been mentioned. Dermographia,[19] urticaria,[19] erythema nodosum[24] and erythema multiforme[13] have called attention to the role of allergy in some cases. Subcutaneous calcification,[25,26] especially over pressure points, occurs almost as often in dermatomyositis as it does in scleroderma. On the other hand, skin changes may remain persistently absent.[11,12]

Edema. Facial edema, especially periorbital,[13] combined with cutaneous telangiectasia is very common in dermatomyositis and has given rise to the popular terms "heliotrope eyelids" and "heliotropic bloating."[14] Swelling may be confined to areas overlying inflamed muscle or may be generalized, tending to become dependent in ambulatory patients. It apparently bears little or no relation to the serum protein changes.[13] Although edema may involve the entire body surface, both intra- and subcutaneously, fluid

collections in the serous cavities are unusual[13] in dermatomyositis.

Myositis. Dermatomyositis can be diagnosed without dermatitis but not without myositis. The term "primary polymyositis" applied to this disease by earlier writers[11] served to emphasize the basic position of the muscular component in this syndrome.

The process may begin in any muscle, and remain limited to one muscle, but it usually involves many muscles in a bilaterally symmetric fashion, with a predilection for the neck,[27] shoulder and pelvic girdle.[28] Pain, weakness and stiffness are the presenting symptoms. Examination may reveal nothing abnormal, or may disclose muscles which are exquisitely tender,[29,30] swollen and doughy. As the disease progresses the muscles may become hard and fibrotic. Lumpy infiltrations and calcium deposits[22] may become palpable.

The tempo of muscle destruction varies greatly. The muscles may be so painful and tender as to make the patient cry out with the least motion or lightest touch. In other instances progressive weakness is the only sign of the insidious atrophy going on. Whether fulminating or gradual, severe loss of muscle tissue results in contractures and deformity[31] reminiscent of that following poliomyelitis. In severe cases the muscles of respiration and deglutition are involved, with death resulting from asphyxia or aspiration pneumonia. Extraocular muscle involvement has been reported.[27] Smooth muscle is never involved but significant myocardial involvement is not unusual.[9,11,13,18,19]

Creatinuria. Ever since Steinitz and Steinfield first recorded creatinuria in a case of dermatomyositis in 1931[30] many investigators have shown that urinary excretion of creatine is consistently and sometimes spectacularly[22] elevated in this disease. It is the most easily measured index of muscle destruction and is thereby the most helpful single laboratory test in the diagnosis of dermatomyositis. The creatine tolerance test (normals retain from 70 to 80 per cent of an oral dose of 1.32 gm. of creatine hydrate),[32] and quantitative analysis of muscle for creatine (normally 400 mg. of creatine per 100 gm. of muscle, decreased to about 200 mg. in muscle of patients with dermatomyositis) afford similar information but are beyond the reach of the average clinical laboratory.

Normal adult men excrete no creatine (up to 200 mg. per twenty-four hours in some laboratories) while women, children, castrates and elderly men may excrete amounts up to 400 mg. per twenty-four hours. Creatine excretion is unaffected by the menstrual cycle,[33] confinement to bed,[34] or by variations normally encountered in the average diet. Increase in the excretion of creatine may be reflected in a corresponding decrease in urinary excretion of creatinine (normally 1 to 2 gm. per day). Some authors attach special significance to the creatinine coefficient[35] (mg. of creatinine excreted in twenty-four hours/kg. body weight, normally 18 to 32; usually much less than 18 mg./kg. body weight in dermatomyositis).

Creatinuria in dermatomyositis may be intense, ranging in various reports as high as 1,100 mg. per twenty-four hours.[11] Creatinuria may also occur in other conditions. Those commonly listed include:[36] (1) Myopathies: muscular dystrophy, the myopathy secondary to amyotrophic lateral sclerosis, myotonia atrophica, amyotonia congenita. (2) Endocrinopathies: diabetes mellitus, thyrotoxicosis, acromegaly, Cushing's syndrome, Addison's disease. (3) Infection: poliomyelitis. (4) Diet: starvation. (5) Drugs: thyroid, ACTH, cortisone and epinephrine.

Scattered reports have mentioned[37] creatinuria in such various conditions as schizophrenia, tetany following parathyroidectomy, high fever of any origin, high protein diet, high carbohydrate diet, dinitrophenol ingestion, toxemia of pregnancy, malignancy, diseases of the liver and diseases of the heart. In these latter conditions creatinuria is rather poorly documented.

The differential diagnostic value of creatinuria in dermatomyositis is enhanced by the fact that creatine excretion is only slight in rheumatoid arthritis[38] and absent in fibrositis.[39] Significant creatinuria may occur in systemic lupus erythematosus,[40] a disease which at times is clinically difficult to distinguish from dermatomyositis. Here, however, the serum L.E. test provides differentiation.

Other Laboratory Findings. An elevated serum globulin is frequent in dermatomyositis. Moderate leukocytosis, 10,000 to 15,000 cells, is fairly frequent. Leukopenia and eosinophilia occur at some time in approximately one-fourth of cases. Anemia is absent or of the mild normochromic variety common to a great host of chronic inflammatory diseases. Elevation of the erythrocyte sedimentation rate is found in 25 per cent of cases.

The urine shows mild albuminuria, up to 2 plus, in about one-half of cases, and is otherwise negative except in very rare instances when severe renal involvement is present.[21]

Other abnormal findings described in isolated reports include low serum sodium, increased serum alkaline phosphatase, diabetic glucose tolerance test, decreased urinary excretion of 17-ketosteroids[40] and decreased peripheral utilization of glucose.[34]

Pathology. The histopathologic changes in the skin in dermatomyositis are not specific[13] enough to make the diagnosis. In the usual case with an erythematous rash, the microscopic findings consist of atrophy of the epidermis with thinning or loss of the dermal papillae, and edema of the basal cell layer with numerous "balloon cells."[41] The dermis and subcutaneous tissue show changes which clearly label the condition as a collagen disease. The collagen fibers undergo degenerative changes, i.e., homogenization, loss of staining ability and thickening.[2] The blood vessels show sclerotic changes in the collagen of their walls, hyaline subintimal proliferations[41] and perivascular cellular infiltrates. It should be noted that the cellular infiltrates in the subcutaneous tissue, around the blood vessels and, as will be noted later, in the muscles, are variable in their composition. The most common infiltrate is lymphocytic but polymorphonuclears, histiocytes and plasma cells are encountered, as well as occasional eosinophils and rare Langhans giant cells.

In the presence of the dermatitides which less commonly appear in the course of dermatomyositis, such as lichen planus,[19,25] eczema, poikiloderma, psoriasis and scleroderma-like patches, the microscopic findings merely mirror the clinical type of dermatitis which happens to be present.[11] However, the collagen and blood vessel alterations already described are basic and will persist to some degree.

The gross pathology of muscle involved by dermatomyositis is limited to abnormal pallor of the muscle and changes in consistency, either mushy or fibrotic and hard. Calcium plaques and hemorrhages are occasionally discernible. The histopathologic changes in muscle include acute degeneration of muscle, with a hyaline appearance due to loss of striations and altered affinity for stains, vacuolation of the cytoplasm, fragmentation and rupture of scattered fibers, blebbing of the sarcolemma sheath and increase in the number of sarcolemma nuclei.[19] The interstitial tissue regularly shows edema, scattered small hemorrhages and cellular infiltrates of varied composition, especially perivascularly. The lesions in cardiac muscle and in the muscles of the tongue and esophagus are identical though usually less profound than those in skeletal muscle.[9] As the disease advances muscle is replaced with fibrous tissue and the end stage is characterized by dense scarring.

Involvement of Other Organs. Although skin and muscle involvement are preeminent in dermatomyositis, this collagen disease like other members of the group may involve practically any organ in the body.

Mucous and serous membranes: Involvement of the conjunctiva[19] has been reported, and approximately one-third of all cases of dermatomyositis have stomatitis[3] the lesions of which include macules, punctate to diffuse erythema, hemorrhages, vesicles, ulcers, leukoplakic patches, pigmentary deposits, edema and scarring. Gingivitis and glossitis are usual accompaniments.

Serositis is unusual in dermatomyositis[42] but there are sporadic reports of pericardial effusion,[31] obliterative pericarditis,[31] pleurisy with effusion,[43] ascites and anasarca.

Cardiovascular system: Raynaud's phenomenon and acrocyanosis[11,21] are fairly frequent manifestations of the widespread arterial involvement in dermatomyositis. In one case differentiation from Buerger's disease was difficult.[44]

The high incidence (approximately 25 per cent) of cardiac involvement in dermatomyositis is perhaps not surprising in view of the fact that the myocardium is striated muscle. Tachycardia out of proportion to the degree of fever, systolic murmurs, pericardial friction rub and auricular fibrillation have been encountered. The most common electrocardiographic abnormalities are low voltage and evidence of nonspecific myocardial damage, both right and left hypertrophy and "strain" having been reported. At autopsy cardiac enlargement and histologic evidence of myocarditis have been repeatedly verified.[9,18,45] Aschoff's nodules are consistently absent.[22]

Respiratory tract: Dermatomyositic destruction of laryngeal and thoracic muscles may produce dysphonia, hoarseness, slowness of speech,[46] dyspnea and difficulty in coughing. Pulmonary infiltrates in dermatomyositis have been described as miliary,[25] hilar[19] and basal linear.[41]

Pathologic evidence as to the nature of these pulmonary lesions is lacking.

Gastrointestinal tract: Gastrointestinal ulceration with hemorrhage and perforation may occur as a result of intimal thickening and thrombosis of small submucosal arteries, anywhere from the esophagus to the rectum.[9] Involvement of striated muscle in the esophagus may cause dysphagia and external anal sphincter may produce fecal incontinence.[46]

Genitourinary tract: Gross hematuria, azotemia[47] and hypertension[42] occur, although rarely, in dermatomyositis.

Central nervous system: Cerebral lesions consisting of petechial hemorrhages,[48] perivascular and interstitial lymphocytic infiltrations,[49] neuronal damage and glial proliferation in the frontal lobes and cranial nerve nuclei[13] have been discovered at autopsy. Disorientation,[41] hallucinations and delirium[19] are encountered clinically. Paresthesias,[21] hypesthesia and lancinating pains have been described, and are attributable to perineural inflammation with demyelinization[13] which can be demonstrated in peripheral nerves. By far the most common "neurologic" abnormality in dermatomyositis, depressed to absent tendon reflexes, is not neurologic at all but simply a result of muscle degeneration and weakness.

Skeletal system: Stiffness and pain on motion, often the earliest symptoms in dermatomyositis, are easily misdiagnosed as arthritis but the incidence of true articular involvement in this disease is only about 5 per cent.[11] In an occasional case the joint process is indistinguishable from rheumatoid arthritis.[49,50] Pathologic study of the joints in dermatomyositis is nowhere to be found in the literature.

Bony involvement in dermatomyositis has been limited to osseous cysts and osteoporosis,[24,31] which may be acute. Milhorat et al.[51] reported decreased urinary excretion of calcium. Serum calcium values have been variously reported as low, normal and high.

Lymphatic system: Generalized lymphadenopathy and splenomegaly[19] have been reported in dermatomyositis. The splenic enlargement may be variable,[13] becoming more pronounced with exacerbations of the disease and receding during periods of quiescence.

Eyes: Diplopia may result when the polymyositis involves extraocular muscles.[27] Iritis occurs rarely[19] but retinopathy with hemorrhages, exudates and pallor of the discs has been detailed repeatedly, together with excellent pathologic studies of the involved eyes.[52]

DIFFERENTIAL DIAGNOSIS

Repeatedly listed in the differential diagnosis for dermatomyositis are such conditions as trichinosis, myasthenia gravis, myositis ossificans, erythema nodosum and Addison's disease. Today the diagnosis of these diseases is a relatively simple matter compared to the differentiation of dermatomyositis from other collagen diseases.

Some considerations of value in delimiting dermatomyositis from the other collagen diseases are as follows:

Systemic Lupus Erythematosus. The dermatitis in dermatomyositis and systemic L.E. is sometimes indistinguishable. Poikiloderma and erythema are the most common skin changes in each disease. In systemic L.E. the "butterfly rash" is said to be pathgnomonic, whereas in dermatomyositis the terms "heliotrope eyelids"[11] and "alabaster facies"[53] have similar import. The variety of dermatitis is much greater in dermatomyositis, in fact it is almost unlimited, ranging from total absence of skin changes to the most spectacular types (ecchymotic, bullous, exfoliative). In both L.E. and dermatomyositis there is depression of 17-ketosteroid excretion, and creatinuria.[40] In dermatomyositis, however, myositis with creatinuria is outstanding, whereas it is minimal in L.E.[54] The serum L.E. test has never been found positive in a case of dermatomyositis.[54] Polyserositis, arthritis, myocarditis and nephritis occur in both diseases but are decidedly more frequent and severe in systemic L.E.[55]

Scleroderma. Thickening and hyalinization of the subcutaneous connective tissue with atrophy of the overlying skin, a not uncommon combination in dermatomyositis, is responsible for the occasional misdiagnosis of scleroderma in patients with dermatomyositis. A few "burned out" cases of dermatomyositis have presented a final picture clinically indistinguishable from scleroderma. Moreover, Raynaud's phenomenon and subcutaneous calcification occur in both diseases. This confusion caused much debate and Osler was prompted to remark that "dermatomyositis is after all a deep seated scleroderma."[56] Distinction between the two diseases is now more clear cut: in dermatomyositis the cardinal characteristic is muscle involvement whereas scleroderma involves chiefly connective tissue, whether it be

subcutaneous, submucosal (esophagus and intestines) or interstitial (lungs and heart). Histologically, many patients with scleroderma show muscle involvement but it is the atrophy of disuse rather than the inflammation and subsequent destruction of muscle which is seen in dermatomyositis.[57]

Periarteritis Nodosa. The close relationship between periarteritis nodosa and dermatomyositis is most evident under the microscope. Perivascular infiltrates, hyalinization of the vascular collagen, intimal proliferation and thromboses of the small arteries occur in both diseases. Eosinophils may be increased in the blood and in the tissue cellular infiltrates in dermatomyositis to further the confusion, although seldom to the degree seen in periarteritis nodosa. Inflammation in the muscles may occur in periarteritis nodosa but it tends to be periarterial instead of the widespread muscle destruction characteristic of dermatomyositis. In periarteritis nodosa skin involvement is unusual. Nephritis, hypertension and neuropathy are common in periarteritis, insignificant in dermatomyositis.

Rheumatoid Arthritis. While involvement of the joints occurs in dermatomyositis, at times mimicking rheumatoid arthritis closely, more commonly there is juxta-articular pain, stiffness and tenderness due to muscular inflammation. Degenerative changes in muscle have been described in rheumatoid arthritis,[58] as has creatinuria. However, there are but faint shadows of the devastation which characterizes dermatomyositis.

ETIOLOGY

Speculation as to the cause of dermatomyositis has run the gamut from protozoal infestation to a dysfunction of the autonomic centers in the floor of the fourth ventricle. Suffice it to say that no single agent has been consistently incriminated and there is reason to believe that dermatomyositis is a syndrome which has diverse causes.

O'Leary and Waisman[11] cultured streptococci and staphylococci from ten of seventeen muscle biopsies and obtained clinical improvement with the use of autogenous vaccines in some cases. Dermatomyositis has appeared so often in patients with tuberculosis[21,30] as to suggest a cause and effect relationship.

In recent years attention has been focused on the remarkable incidence of carcinoma in patients with dermatomyositis. Carcinoma developed in eight of forty-five patients with dermatomyositis (18 per cent), according to Curtis et al.[59] These authors collected twenty-seven similar cases from the literature and we found sixteen additional cases[12,22,26,27,46,60,61,62] reported since their article was published, for a total of fifty-one such "coincidences." No particular type of cancer is incriminated, those reported include (in order of frequency): neoplasm of the ovaries, breast, reticulo-endothelial system, stomach, gallbladder, cervix, rectum, esophagus, lung, kidney, pituitary, parotid, bone and retroperitoneum. Of special interest is the improvement in the dermatomyositis which has been observed to follow treatment of the neoplasm, whether by irradiation or surgery.

Although the exact relationship of cancer to dermatomyositis is not clear, the hypothesis has been offered that dermatomyositis may arise on the basis of an allergic reaction to protein breakdown products of the tumor. There is also increasing support for the view that in patients with dermatomyositis sensitization to a variety of antigens such as the tubercle bacillus, streptococci or their products, may be the basic pathogenetic mechanism. This is in line with the current concept that the etiologic common denominator in the collagen diseases is the vascular "hypersensitivity" reaction described by Rich.[63]

PROGNOSIS

Dermatomyositis is a more severe disease with a higher mortality during the first few decades of life when muscular development and activity are at a high level. Fulminating cases with death in six to eight weeks are rare, occurring only in children, the usual case running its course in an average of two years.[30] The over-all mortality rate is 50 per cent with considerable morbidity among the survivors in the form of fibrotic muscular atrophy. Spontaneous remissions may last as long as eight years,[11] which makes evaluation of treatment uncertain. The most common cause of death is aspiration pneumonia initiated by weakness of the muscles of deglutition and respiration. Fatal termination may also be produced by myocarditis, intestinal perforation and uremia.

TREATMENT

ACTH and cortisone abolish the inflammatory manifestations of dermatomyositis (fever,

tachycardia, erythema, leukocytosis and elevated erythrocyte sedimentation rate)[64] but this is a non-specific effect, even though it may be of enormous temporary benefit to a prostrated "toxic" patient. More specific improvement has been reported, i.e., reduction of creatine excretion,[65] increase in muscular strength and conversion of muscle biopsy to normal.[66] Prolonged remissions have been induced with steroid therapy but in some patients relapse promptly follows withdrawal of the hormones.[67]

Reduction in creatine excretion and a return toward normal of muscle consistency to palpation and of muscle strength in patients with dermatomyositis have been reported to follow the use of wheat germ oil and tocopherols.[23]

The protein anabolic action of testosterone has led to the trial of this hormone in dermatomyositis, a muscle-wasting disease. Sustained remissions have followed its use[68] but evidence of its value cannot be classified as more than a clinical impression. Thorn et al.[69] recommend the use of testosterone to maintain control of dermatomyositis once a remission has been induced with ACTH and cortisone.

SUMMARY

Two cases of dermatomyositis are described. One patient had severe muscle atrophy, arthritis with contractures, dependent edema and pleuropulmonary involvement. Dermatitis remained absent. The second patient presented psoriasiform dermatitis which became exfoliative, generalized edema and evidence of cardiac involvement.

Striking creatinuria was present in both patients and its diagnostic value is emphasized.

Gastric ulcers with hematemesis developed in one patient and acute psychosis in another, complications which may have been due either to ACTH-cortisone therapy or to dermatomyositic involvement of the stomach and brain, respectively.

A review of the literature reveals that dermatomyositis has protean manifestations but can nonetheless be clinically differentiated from the other collagen diseases. To date fifty-one instances of carcinoma and dermatomyositis occurring in the same patient have been reported. It has been suggested that the incidence of carcinoma in these patients is more than coincidental, and that search for an occult neoplasm is indicated in every patient with dermatomyositis.

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Whipple's Disease*

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IN 1907 G. H. Whipple[28] wrote of "a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal tissue and mesenteric lymphatic vessels" and characterized clinically "by gradual loss of weight and strength, stools consisting chiefly of fat and fatty acids, indefinite abdominal signs and a peculiar multiple arthritis." He described the large pale macrophages which gave a negative reaction to fat stains and told of the polyserositis. Thus he recorded all of the outstanding characteristics of the entity which bears his name.

Sixteen years elapsed before the next reported case,[3] and a total of only six cases had been reported by 1931. With increasing awareness of this entity by clinician, surgeon and pathologist, fifty-three cases have been reported since 1931, and diagnosis by laparotomy and biopsy, first recorded in 1947,[18] has now become the rule. Ante mortem diagnosis has resulted in additional investigation and treatment, and recent results of treatment with ACTH and cortisone[7,13,23,24] have been encouraging in this otherwise fatal condition.

Because of the prominent lipid collections in the intestinal mucosa and mesenteric lymph nodes, the terms "lipogranulomatosis" and "intestinal lipodystrophy" have become ingrained in the literature. Although anatomically descriptive, the etiologic implications of these terms seem to be in error. The most characteristic elements of the lesion are the collections of sudan-negative macrophages in the lymph nodes and in the tunica propria of the mucosa of the small bowel. Black-Schaffer[2] demonstrated that the material within these cells reacted positively to the periodic acid Schiff test, and his studies led him to the conclusion that this material was a glycoprotein. Histochemical studies made by Upton[25] resulted in the same conclusion. Of further interest in this regard was the demonstration of an abnormal amount of glycoprotein in the serum of the one patient tested.[24] Although of help in reorienting etiologic concepts, these observations have not resulted in establishment of the fundamental etiology. The arthritis and polyserositis, the course and variability of the illness, the occasional occurrence of endocarditis and vascular lesions and more recently the beneficial results of ACTH and/or adrenocorticosteroid therapy have all brought support to Clemmesen's suggestion[5] that this is another entity in the group of "rheumatic" or "collagen system" disorders. Aschoff bodies[12] and "wire-loop" deformities[20] have each been demonstrated in patients with this disease on one occasion. In investigating this relationship further Jones et al.[13] were not able to demonstrate any PAS-positive material in the cells of the small intestines of a few patients with scleroderma, rheumatoid arthritis or disseminated lupus erythematosus. Other observers have suggested that faulty absorption of abnormal mucin (possibly produced by the intestinal mucosa) results in a generalized allergic reaction as well as phagocytosis of the material by the regional reticuloendothelial cells.[2,25] The latter proposal has the advantage of "explaining" the most distinctive element of the disorder, its distribution, and the systemic features of the disease.

To date, a total of fifty-nine cases[1,4,8,10,15,22–24,26] have been reported as Whipple's disease. Some of these diagnoses have been in error. At present, the only safe criterion for diagnosis would appear to be the characteristic PAS-positive macrophage in the typical distribution, regardless of other findings.

The authors wish to record four more instances of this entity to emphasize the diagnostic value of peripheral node involvement, to report the occurrence of the disorder in siblings and to comment on the possible role of genetics in the etiology. No attempt will be made to give complete anatomic descriptions of the lesions but enough data will be given to document the cases.

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CASE REPORTS

CASE 1. C. S., a forty-three year old, white, single, Canadian man employed as a chrome plater, was first seen at The Henry Ford Hospital in September, 1943. The chief complaints were of abdominal cramps and diarrhea of three weeks' duration. The patient considered his previous general condition to have been good. He had had migratory polyarthritis of unstated duration which had cleared prior to the onset of diarrhea. He denied any venereal disease. The right ankle had been fractured in 1928. He suffered from urticaria "when overheated." He had used alcohol freely until five weeks prior to admission. He had a mild chronic cough, suffered frequent "colds" and had occasional "hemoptysis," the latter being attributed to the acid used at his place of employment. He had also had frequent night sweats.

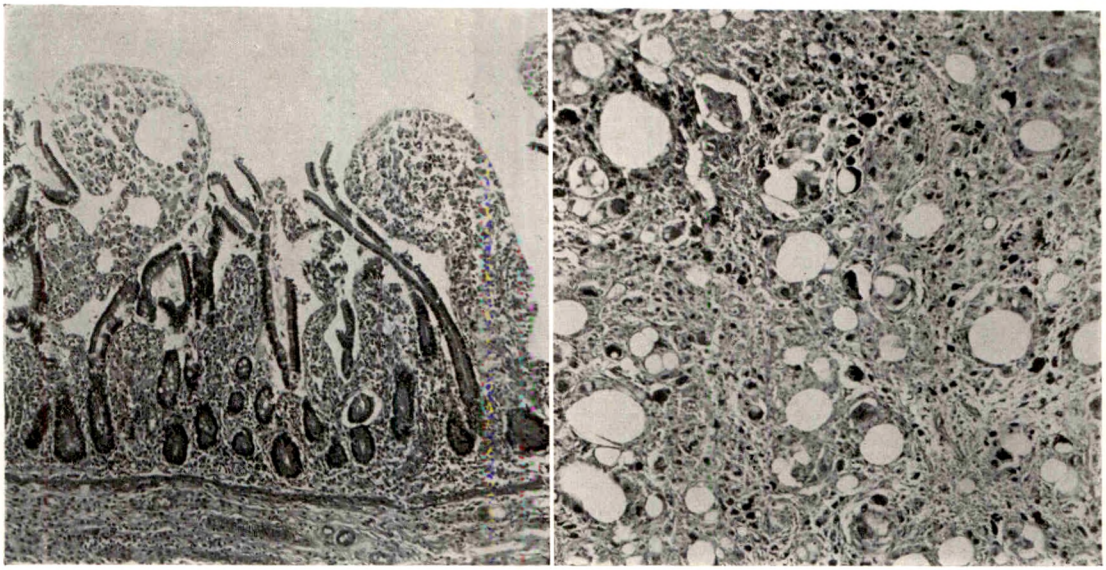
With the present illness the patient had lost 18 pounds. He had experienced nausea, occasional vomiting and attacks of generalized abdominal cramps. He had numerous watery, tan, malodorous bowel movements without mucus or pus, but some blood had been noted during the previous four days. Appetite remained good. Vomiting occurred three to four hours after meals and followed generalized abdominal cramps. The vomiting was unrelated to fatty food intake.

Physical examination revealed a thin, well developed, white man in no apparent distress. His temperature was 98.6°F., pulse 80, respiration 20 and blood pressure 110/68. There were upper and lower partial dental plates. "Pyorrhea" was marked and oral hygiene was poor. No abnormal pigmentation was noted. Cardiac examination gave normal results except for soft apical and basal systolic murmurs. A herniorrhaphy scar was present in the right inguinal region, and slight inguinal adenopathy was present bilaterally. Abdominal, neurologic, genital, rectal and musculoskeletal examinations gave normal results.

Proctoscopic examination revealed ulcers from pinpoint to 1 mm. in diameter throughout the examined region; surrounding mucosa was normal. Several weeks later this was not present. Hemoglobin was 7.4 gm. per cent, white blood count 15,300 mm.^{3}, urine specific gravity 1.032 and otherwise normal. Kline exclusion and cephalin-cholesterol tests gave negative results. Examination further revealed normal fasting free HCl and normal response to histamine on gastric analysis, normal duodenal drainage except that enzymatic studies were not done and a barium enema gave normal results. Fluoroscopically the gastrointestinal series showed spasm of the antrum and a possible ulcerating lesion on the lesser curvature of the stomach that was not demonstrated on the films.

Discharged on an ulcer regimen, the patient returned ten days later with severe abdominal cramps and distention. Abdominal x-ray showed considerable gas in the large bowel. Symptoms now included pain in the back immediately after meals. The patient's temperature was 99.2°F. and pulse 80/min. Additional observations included a distended tympanitic abdomen with moderate generalized tenderness and lymphadenopathy in the supraclavicular, axillary and inguinal regions. Hemoglobin was 7.7 gm. per cent, red blood count 3.5 M/mm.^{3}, white blood count 11,300/mm.^{3}, neutrophils 78 per cent, lymphocytes 19 per cent and eosinophils 3 per cent. Urine was normal except for 22.5 mg. of albumin/100 cc. He developed a daily afternoon fever which reached a maximum of 102°F. every fifth day. White blood cells ranged from 11,300 to 17,840/mm.^{3} with differential ranges of neutrophils 78 to 87 per cent, lymphocytes 9 to 19 per cent and eosinophils 1 to 4 per cent. An axillary node biopsy and later, a laparotomy were undertaken, the latter revealing considerable clear fluid in the abdomen; the mesenteric lymph nodes were found to be greatly enlarged and rather firm. One of these was removed. The total protein of the abdominal fluid was 1.5 gm. per cent. Plasma protein at this time was 3.9 gm. per cent. Following surgery the patient symptomatically improved and was afebrile for eight days. He received x-ray therapy, thereafter developed severe nausea, returned to his previous febrile pattern which progressed to daily spikes to 103°F. and again developed diarrhea with rapidly progressive inanition. Without the slightest response to therapy the patient died on the fifty-fifth postoperative day.

Autopsy revealed the body to be undernourished and there were numerous petechial hemorrhages in the skin. Both the left portion of the pleural cavity and the pericardial sac were obliterated by dense fibrous adhesions. Moderate pulmonary edema and pneumonia was present. The main findings were in the



1 2

FIG. 1. Case 1 (C. S.), section from jejunum; villi show clubbing due to accumulation of PAS-positive macrophages; hematoxylin and eosin stain.

FIG. 2. Case 1 (C. S.), section from mesenteric node; black cells represent PAS-positive macrophages. Note fat vacuoles surrounded, in many instances, by foam cells and giant cells; periodic acid Schiff stain.

abdomen. It contained about four liters of clear yellow fluid, and the mesentery of the small bowel was thickened and attached to the retroperitoneal mass. The mass was composed of enlarged lymph nodes which were yellow-orange, firm and honeycombed on cut section. The bowel showed only some thickening of the rugal folds. The liver was slightly enlarged with fibrous thickening of its capsule.

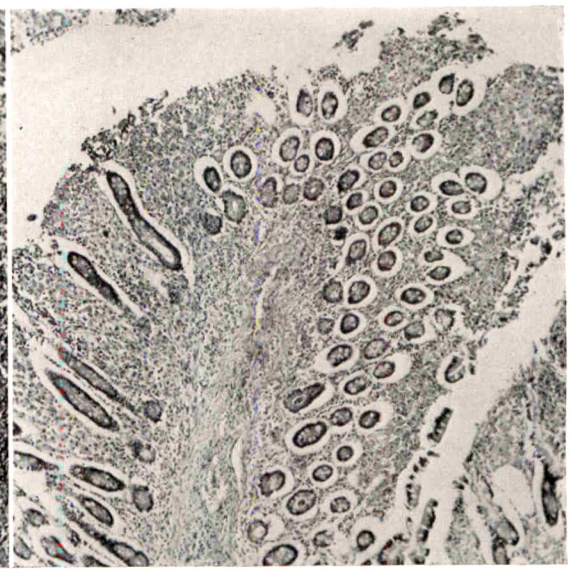
Study of the sections showed characteristic change. The small bowel had thickened villi due to accumulation of PAS-positive macrophages. (Fig. 1.) There was also edema and moderate infiltration of the submucosa with these macrophages. The mesenteric nodes also showed typical nests of PAS-staining macrophages and fat vacuoles eliciting a foreign body reaction. (Fig. 2.) In peripheral nodes the characteristic masses of PAS-positive cells were also present. (Fig. 3.) Retroperitoneal connective tissues in general contained numerous PAS-positive cells scattered diffusely. Nests were also seen in the portal areas in the liver.

CASE II. P. V., a twenty-seven year old married Lithuanian dairy farmer, was first admitted to the Henry Ford Hospital in October, 1944. The chief complaints were of "inflammatory rheumatism" of two to three years' duration and "epigastric pain" of one year's duration. At age sixteen he had had difficulty for two months in raising either arm to the horizontal position. This was diagnosed as "rheumatism" and cleared spontaneously. A bullet was excised from the mandible at age twenty. At age twenty-one he suffered "head and internal injuries" in an automobile accident and was disabled for nine months.

Two to three years prior to admission he had developed a polyarticular migratory joint disturbance with swelling, tenderness and pain. This had been quiescent for some months prior to hospitalization. For several years he had also been subject to mild attacks of cramping abdominal pain. During the preceding year these attacks had been much more severe. Initially the pain had been dull and aching, had been localized over the anterior lower portion of the chest and upper portion of the abdomen bilaterally, had radiated to the left shoulder and dorsal spine. These attacks usually occurred once every ten to fourteen days and lasted several days. The distress was aggravated by jarring, lifting, sneezing, coughing and particularly by deep respirations. Recent cramping in the upper portion of the abdomen had been severe and at its worst one-half hour after eating. There was mild, dull pain in both costovertebral angles with no associated urinary symptoms



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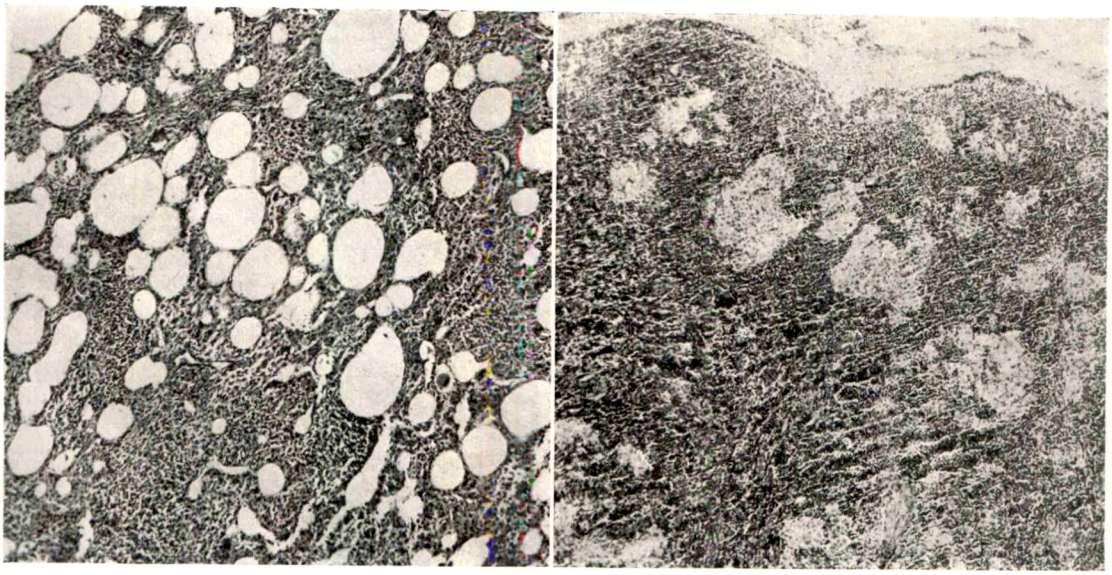
FIG. 3. Case I (C. S.), section from axillary node; numerous nests of macrophages in the cortex filled with PAS-positive material; hematoxylin and eosin stain.

FIG. 4. Case II (P. V.), section from jejunum; stroma of mucosa filled with plump macrophages; hematoxylin and eosin stain.

other than nocturia (one to two times) for the preceding four months. He was subject to slight shaking chills followed by slight fever and had lost 27 pounds in the preceeding six months. He drank unpasteurized milk and had had contact with "Bang's" disease. Tests for brucellosis previously had given negative results.

Physical examination revealed an asthenic, pale, thin youth who complained of abdominal pain. His temperature was 99.6°F., pulse 72/min., respiration 16/min. and blood pressure 100/60 mm. Hg. The skin was warm and moist and had a "grayish" appearance. The teeth contained many fillings. Lymphadenopathy was marked in the left axilla and "shotty" in the inguinal and cervical regions. The abdomen was tympanitic and "resistant," particularly on the left side. Pressure on a point of maximal tenderness located 4 cm. below and to the left of the umbilicus caused pain in the right costovertebral angle. Several examiners thought a mass was palpable in the left lower quadrant of the abdomen. The left testicle was slightly enlarged and there were small bilateral hydroceles. Motion of the back was restricted in all directions and there was tenderness on percussion over the lower dorsal portion of the spine.

Laboratory examinations revealed the hemoglobin to be 13.5 gm. per cent, red blood count 4.25 M/mm.^{3}, white blood count, 13,850/mm.^{3}, neutrophils 81 per cent, lymphocytes 13 per cent, eosinophils 6 per cent, sedimentation rate (Wintrobe) 6 mm./hour, serum non-protein nitrogen 33 mg. per cent, fasting blood sugar, 58 and 94 mg. per cent and serum diastase was normal. Repeated urinalysis revealed only a trace of albumin on one occasion and this specimen was negative for Bence-Jones protein; urine cultures gave negative results. Cystoscopic examination and intravenous and retrograde pyelograms were within normal limits. Biliary drainage (exclusive of enzyme studies) was normal. Fractional gastric analysis showed only two units of free HCl on the second specimen after histamine. Proctoscopic examination, gastric, colonic and small bowel x-ray studies after barium, and cholecystographic studies gave normal results. Slight thickening in the interlobar fissure on the right side was revealed by chest x-ray. X-rays of the spine were within normal limits. Blood cultures gave repeatedly negative results. Stools were consistently 3 to 4 plus guaiac-positive and on one occasion amebic cysts were seen. Agglutinations for Brucella abortus, typhoid, paratyphoid and dysentery, complement fixation and phagocytic index for Br. abortus, and skin test for brucellosis and trichinosis all gave negative



5 6

FIG. 5. Case II (P. V.), section from mesenteric node; numerous fat vacuoles. PAS-positive macrophages are scattered through the stroma but are not easily seen; hematoxylin and eosin stain.

FIG. 6. Case III (E. V.), section from axillary node. Cortical nests of PAS-positive cells again well demonstrated; hematoxylin and eosin stain.

results. The Kline exclusion test gave negative results.

During hospitalization the patient ran an afternoon fever each day and this reached its maximum of 101°F. to 101.4°F. every five to six days. Abdominal pain and episodes of chills continued and he developed diarrhea. Hemoglobin dropped slowly to 10 gm. per cent, the white blood count remained around 9000/mm.^{3} with no significant change from the differential previously cited. Repeated sedimentation rates were always over 30 mm./hour.

Laparotomy revealed thickening of the wall of the small bowel, thickening and induration of the mesentery, and numerous firm, enlarged mesenteric lymph nodes, one of which was removed for microscopic examination.

The patient received x-ray therapy to the abdomen and supportive therapy. He was able to return home where he continued to be febrile, to have severe episodes of abdominal pain that would "double him up," and to have periodic chills, pleurisy and diarrhea. He refused to eat because this aggravated his distress, and continued to lose weight. Pigmentation increased. Diarrhea became profuse and terminally hemorrhagic. The patient died in another hospital two months later.

At autopsy, numerous hemorrhagic nodules were seen in the skin of the legs and in the intestine and kidneys. The mesenteric nodes were markedly enlarged and the cut surfaces were tan-orange.* Microscopic studies were limited to hematoxylin and eosin sections. The changes in the small bowel and in the mesenteric lymph nodes were again characteristic. They showed clubbing of the villi in the bowel due to large numbers of macrophages with abundant pale cytoplasm. (Fig. 4.) The mesenteric nodes showed both lipid accumulation and macrophages. (Fig. 5.)

CASE III. E. V., a thirty-five year old Lithuanian man employed as a factory worker, was admitted to the Henry Ford Hospital in August 1953. The chief complaints were of chills, fever, weight loss and diarrhea. A compound fracture of the tibia and fibula in the lower third portion of the right leg in 1939 had resulted in an unstable fibrous union which was repaired by bone graft in 1943. At this time there was a "branny macular type of eruption on the chest, back and buttocks." Blood pressure was 120/80 mm. Hg, hemoglobin 16.3 gm. per cent and other studies gave normal results. Eight months later a draining sinus developed. A generalized urticarial reaction followed a

* Data were obtained with the cooperation of Dr. G. W. Ramsey, Washington Hospital, Washington, Pa.

course of penicillin in beeswax. Sporadic treatment failed until healing was secured by saucerization and local streptomycin therapy in 1946. Hemoglobin was 13 gm. per cent, and other blood studies including erythrocyte sedimentation rate and urinalysis gave normal results at this time. The patient had gonococcal urethritis in 1946. In 1948 he developed a red papular pruritic lesion over the area previously described soon after beginning phenobarbital therapy, to which the rash was attributed. He also developed pretibial nodules. Both disappeared spontaneously without cessation of phenobarbital therapy. It was also in 1948 that the patient first developed a vague "hunger-type" abdominal pain localized in the periumbilical region which occurred sporadically and was unrelated to eating.

In 1951 the patient was admitted to the hospital because of painful, hot, swollen and tender joints of two and one-half years' duration. All of the larger joints had been involved at some time or another during this period. He had lost 20 pounds. The subject had had chilling sensations, attacks of pleurisy, a mild productive cough and night sweats. At this time abdominal distress was mainly postprandial. Examination revea'ed a tender, hot, swollen right ankle, tender nodes in the inguinal regions, "shotty" nodes in the axillae, and blood pressure of 105/60 mm. Hg.

Laboratory examination revealed the hemoglobin to be 12.3 gm. per cent, red blood count 4.11 M/mm.^{3}, neutrophils 63 per cent, lymphoyctes 30 per cent, eosinophils 7 per cent, non-protein nitrogen 38 mg. per cent, sedimentation rate 25 mm./hour (Wintrobe), serum uric acid 2.8 mg. per cent and urinalysis gave normal results. Electrocardiogram, intravenous pyelogram and retrograde pyelograms were within normal limits. Spine films revealed "fusion of the sacro-iliac joints indicative of an old Marie-Strümpell's arthritis." The patient received bile salts and salicylates at this time.

In 1952 a left epididymitis and orchitis developed which partially cleared during four weeks of aureomycin and sulfadiazine therapy only to recur on the right side. This too subsided. However, it was necessary to excise a non-painful, well circumscribed nodule ½ by 2 cm. in diameter from the anterior upper pole of the left testicle. Unfortunately, no tissue reached the laboratory. Cystoscopic examination and intravenous and retrograde pyelography were within normal limits. Reports of cultures and smears of the urine are not available. Film of the chest showed a small linear density on the right which was not thought to be significant. Blood studies at this time were not significantly different from those in 1951, except for a white cell count of 12,250/mm.^{3}

The patient gradually lost energy and initiative and became easily fatigued and irritable. Recurrent attacks of pleurisy, usually in the lower left anterior portion of the chest and radiating to the left shoulder, became more frequent and lasted three to seven days. Attacks of abdominal pain were more severe and were usually experienced in conjunction with chills, fever and profuse sweating. In addition to the aggravation caused by eating, there was a persistent dull generalized abdominal aching, and the subject was comfortable only when standing erect or when leaning far forward in the sitting position. He was also extremely uncomfortable for a short time after lying down. He became persistently nauseated but there was no vomiting, constipation, diarrhea or melena. All arthritis had subsided seven months before this admission in 1953.

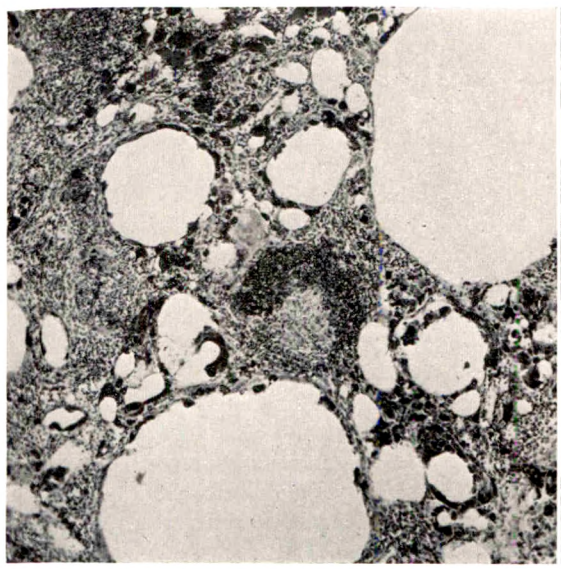
Physical examination revealed a chronically ill white man. His weight was 140 pounds, height 6 feet, temperature 99.4°F., pulse 76/minute and regular, respiration 20/minute and blood pressure 110/68 mm. Hg. His emaciation and general skin pigmentation were outstanding. The latter was particularly prominent over bony prominences and pressure areas but did not involve the oral mucosa. Lymphadenopathy consisted of small anterior and soft, flattened, pea-sized posterior cervical and supraclavicular nodes, several 2 by 1 cm. axillary nodes, and soft conglomerate nodes of the same size in the inguinal regions. He was edentulate. The abdomen had a "doughy" consistency. The liver was 4 cm. below the costal margin in the midclavicular line. In the left epigastrium there was a poorly defined tender mass descending on inspiration. The abdomen was hyperresonant except in the left hypochondrium. Peristalsis was decreased. There was three-plus rebound and localized tenderness at any abdominal area stimulated. Although there was a history of slight ankle edema and aphthous stomatitis, there was none at this time. A pleural friction rub was heard during an attack of pleurisy.

Laboratory examination revealed the hemoglobin to be 9.5 gm. per cent; red blood count

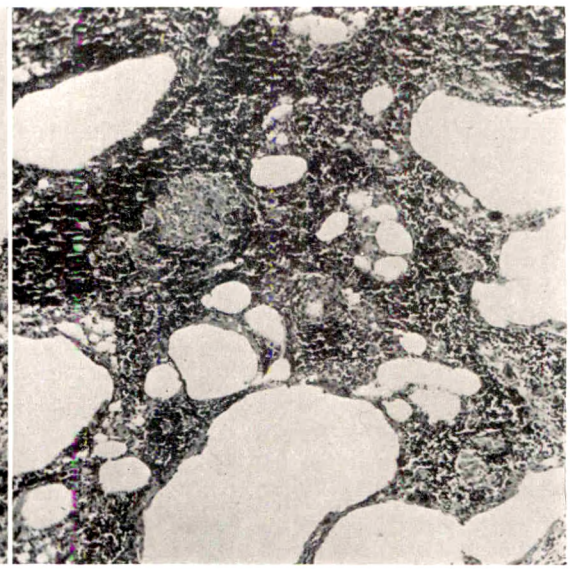
4.09 million/mm.^{3}; white blood count 20,000/mm.^{3}; differential, 90 per cent neutrophils, 7 per cent lymphocytes, and 3 per cent eosinophils; bone marrow myeloid/erythroid ratio 5.6/1 (normal 3/1 or 4/1) with 15 per cent eosinophils; serum non-protein nitrogen 35 mg. per cent; blood urea nitrogen 13 mg. per cent; creatinine 0.7 mg. per cent; uric acid 2 mg. per cent; albumin 4.4 gm. per cent; globulin 2.9 gm. per cent; Van den Bergh direct 1.0 mg. per cent and total 2.0 mg. per cent; thymol turbidity test 1 unit; cephalin and thymol flocculation tests negative results; diastase 69 per cent; prothrombin time 15 seconds or 80 per cent; cholesterol 241 mg. per cent with 48 per cent esters; serum glycoproteins 15.7 and 8.2 mg. per cent tyrosine per 100 cc. (normal 2.5 mg. per cent); sodium 139 mEq./L.; potassium 4.3 mEq./L.; calcium 9.8 mg. per cent; phosphorus 4.2 mg. per cent; CO_2, 23 mEq./L.; chloride 98 mEq./L.; alkaline phosphatase 2.2 Bodansky units; eight hour intravenous ACTH tests normal results on two occasions; total direct eosinophil counts 934, 576 and 1546; tests for cryoglobulinemia negative results; urine negative for Bence-Jones protein on three occasions; Coombs' test negative results on two occasions; heterophil agglutinations, cold agglutinations, Widal test, brucella agglutinations, complement fixations and phagocytic indices, Wassermann test, Weil-Felix test, and P. tularensis agglutinations all gave negative results. Skin tests including first and second strength purified protein derivative of tuberculin, the Frei test, and the histoplasmin test gave negative results. Urine cultures for acid-fast bacilli gave repeatedly negative results. The only free hydrochloric acid obtained by gastric analysis was on the third specimen after histamine. The results of the oral glucose tolerance were: fasting 85, 30 minutes 172, 90 minutes 129 and 150 minutes 120 mg. per cent. BSP test resulted in 4 per cent retention at 45 minutes and 3.5 per cent retention at 60 minutes after injection. A seventy-two hour stool specimen by dry weight contained 47.3 per cent fat. Barium enema, chest, skull and pelvic x-ray examinations gave normal results. Upper gastrointestinal x-ray examination revealed a "deficiency pattern" in the small bowel. An axillary and inguinal node were removed; the changes are more fully described later. Among other suggestions, the possibility of Whipple's disease was mentioned.

While in the hospital the patient ran an intermittent undulating febrile course, the highest temperature being reached every fifth to seventh day at about 101°F. There were shaking chills and drenching night sweats, most marked when the temperature was maximal. He refused food, was deteriorating rapidly, and death seemed imminent within days. A laparotomy under spinal anesthesia revealed a lusterless peritoneum and a large, rather firm, nodular tumor mass between the leaves of the mesentery and extending into the retroperitoneal space. Additional similar tissue was found along the lesser curvature of the stomach. Both tissues were biopsied. The small bowel wall was thick and edematous but not otherwise unusual.

Following surgery the patient made an uneventful recovery and was afebrile and relatively asymptomatic for about sixteen days when all symptoms and findings recurred. One hundred mg. of cortisone per day over a two-week period had little effect on the fever or symptoms. Within four days from the beginning of subcutaneous administration of 100 units per day of ACTH gel, he was afebrile and asymptomatic. Slight physical overexertion would result in the return of both within a few hours. On this therapy alone the hemoglobin rose rapidly to 13.5 gm. per cent. For more complete therapy, folic acid, parenteral vitamin B_{12} in massive doses, bile salts, emulsifying agents, pancreatin and multivitamins were then added. He gained weight to 170 pounds without edema or other evidence of hypercorticism. The blood pressure was consistently about 110/60 mm. Hg. After two months he began to have cycles of fever and had a recurrence of symptoms of gradually increasing severity. Pigmentation constantly increased. Dosage was gradually decreased and, except for a temporary episode of arthritis and erythema nodosum, the condition did not become worse except when less than 40 units a day was given. On 100 mg. of compound F per day, given orally, the subject again experienced a remission. Pigmentation decreased markedly and even the recent brown-black scar cleared almost completely. However, a profuse diarrhea developed which was then controlled by diet, oral streptomycin and a return to ACTH gel at 80 units per day. He continued to lose weight, a cough of increasing intensity developed and he became extremely weak despite all measures. Pneumonia of the right lower lobe developed three days prior to death and was followed by abscess formation. No specific organism could be



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FIG. 7. Case III (E. V.), section from mesenteric node. In the lymphatic nodule in the center there is a nest of macrophages which gave negative results with Sudan stains but positive results for a PAS stain. Numerous fat laden cells can be visualized. Sudan III stain and hematoxylin and eosin stain.

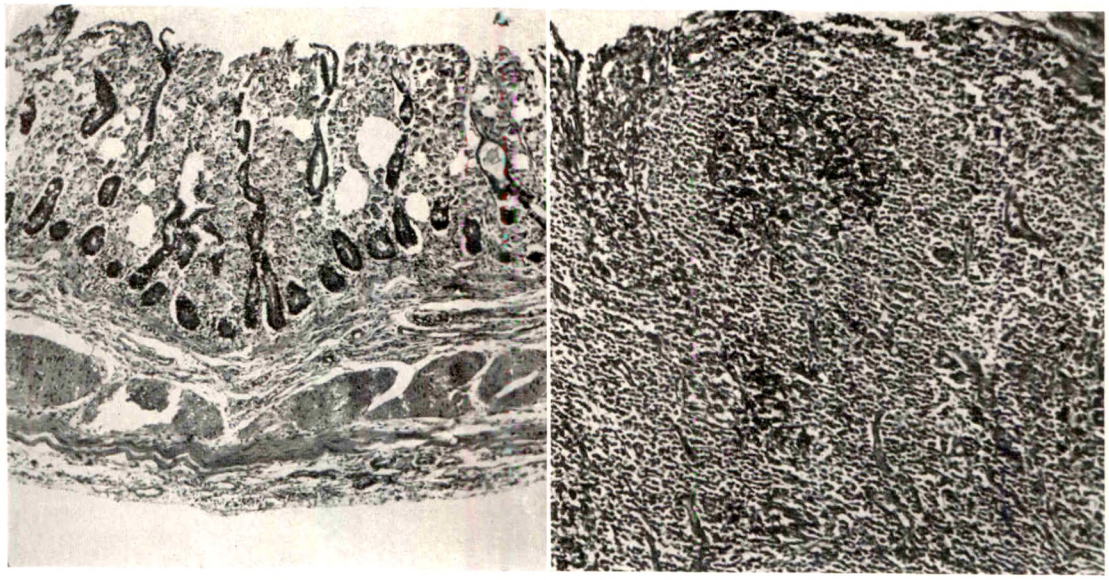
FIG. 8. Case III (E. V.), same as Figure 7 except for hematoxylin and eosin stain. The two types of macrophages may be difficult to distinguish without special stains.

identified and vigorous parenteral penicillin and streptomycin treatment was without effect. Temperature was sustained at 103 to 104°F. Platelet count and prothrombin time were within normal limits and hemoglobin was 12.5 gm. per cent the day prior to death. Acute shock developed one-half hour before death and did not respond to vasoconstrictor agents. The patient became comatose and died. Electrocardiograms the day before and twenty minutes prior to death showed inversion of T waves in leads V_3, V_4, V_5, but no evidence of electrolyte disturbance.

The cervical and inguinal nodes removed at surgery were moderately enlarged and pale. Study of the sections showed numerous nests of large pale macrophages scattered about, especially in the cortical portion. These showed fairly abundant pale cytoplasm and were PAS-positive. Germinal centers were small or absent. Some proliferation of reticuloendothelial cells and a mild amount of central fibrosis were present. (Fig. 6.) The axillary node showed a similar picture. Bacteriologic studies of material from these nodes failed to show specific organisms. The mesenteric node was enlarged, tan, firm and showed a fine honeycombing on cut surface. The section showed many characteristic nests of the PAS-positive macrophages plus many fat vacuoles. The latter were often surrounded by foam macrophages which were Sudan-positive. (Figs. 7 and 8.) Microscopic examination of the liver biopsy gave normal findings.

At autopsy the skin showed some increased pigmentation. About 200 cc. of slight turbid fluid were present in the abdomen and about 300 cc. in the right chest. The small bowel was distended with gas and injected. The mesentery was indurated and contained many enlarged tan, honeycombed lymph nodes. Dilated lymphatics were not seen. There was serositis of the liver and spleen and an adhesive pericarditis was present. Lobar pneumonia was present in the right lower lobe in the stage of gray hepatization. The mucosa of the small bowel was congested and several patches showed a granular pallor and a thickening of the plicae. The adrenals were small with atrophic cortices. Microscopic studies showed the characteristic macrophages in the mucosa of the small bowel (Fig. 9) and in the lymph nodes from mesentery and all peripheral areas.

Familial History of Cases P. V. and E. V. (Fig. 11.) The father of the patients in Cases II and III was living and in good health. No information was available as to the cause of death of the father's



9 10

FIG. 9. Case III (E. V.), section from jejunum. The PAS-positive macrophages in the stroma of the mucosa are well shown; hematoxylin and eosin stain.

FIG. 10. Case IV (W. McC.), section from axillary node. A large nest of PAS-positive cells can be observed near the periphery, as well as several other cells which are scattered; periodic acid Schiff stain.

parents. The remainder of his relatives were in good health with the exception of a nephew who had rheumatoid arthritis. The maternal grandfather was killed in an accident. The grandmother died at the age of sixty-three following a year's illness characterized by abdominal pain, diarrhea, emaciation and terminal hemorrhagic diarrhea. She is not known to have had arthritis.

According to the family there was no doubt that the patient's mother had died of the same disease as did the two sons. She had suffered for several years with a migratory polyarthritis characterized by hot, tender swollen joints which had been diagnosed as rheumatoid arthritis. With the onset of subsequent symptoms this disappeared. About one year prior to death she developed a prolonged upper respiratory infection. Hospital records state that she then began experiencing chills intermittently. On one occasion she had been suddenly seized with pain on deep breathing, chills, fever and marked perspiration, and had been hospitalized. She was an obese woman, was edentulate and febrile (102°F.) with a pulse of 120/minute and respirations of 40/minute. Numerous moist rales were heard on inspiration and expiration and a friction rub was heard on the right side posteriorly. No hemoptysis or congestive failure was evident. There were frequent extrasystoles. Studies revealed exaggeration of "parabronchial markings on the left" by chest x-ray, red blood count was 3.6 million/mm.^{3}, hemoglobin 71 per cent, white blood count 8.900/mm.^{3}, serum nonprotein nitrogen 27 mg. per cent and negative results were obtained from sputum cultures, acid-fast smears and serological tests for syphilis. On sulfadiazine therapy the temperature became normal after three days but then returned to 102°F. This temperature recurred several times during this twenty-day hospitalization. Following discharge she developed abdominal pain which later became severe and cramping in nature and was precipitated by eating. She developed increasingly severe diarrhea and meteorism. Weight loss was rapid. Eight months after the previous hospitalization studies revealed a trace of albumin by urinalysis, hemoglobin 51 per cent, red blood count 3.1 million/mm.^{3}, white blood count 5,500/mm.^{3}, neutrophils 74 per cent, lymphocytes 26 per cent and Kahn and Kline tests gave negative results. X-ray examination revealed a normal stomach and duodenum. Six hours after barium meal the small bowel was empty except for "a few scattered flecks," and the cecum somewhat conical in shape. Barium enema showed the cecum not adequately filled, the appendix was visualized and there was no reflux into the

terminal ileum; the remainder of the colon was normal. Postevacuation films were thought to show some increased density "apparently of soft tissue origin" over the right sacroiliac area. She became increasingly pigmented and emaciated. Terminally, the diarrhea became hemorrhagic and she died two and one-half months after the examinations described.

Of the mother's two siblings, a sister is living, has three children (two girls, one boy) and all are apparently well. The other, a brother, was institutionalized at the age of thirty-one following seven years of gradual mental deterioration during which time he was unable to support himself. He was apparently schizophrenic. At age thirty-two blood studies gave normal results except for slight leukocytosis. The serum non-protein nitrogen was 30 mg. per cent and chest x-rays each year were within normal limits until age thirty-five when slight cardiac enlargement was noted. A year later a right pleural effusion was noted. Two months later this had completely cleared. The red blood count was 3.7 million/mm.^{3}, hemoglobin 70 per cent, serum non-protein nitrogen 30 mg. per cent and fasting blood sugar 55 mg. per cent. Several urinalyses showed only numerous bacteria. Two months later enlargement at the hilum, thought to represent enlarged lymph nodes, was apparent. Some cardiac hypertrophy was still present. After another two months the serum non-protein nitrogen was 42 mg. per cent and chest x-ray showed interlobar effusion and effusion extending over "the entire periphery" of the left lung. Bilateral hilar enlargement remained. The sedimentation rate was 27 mm./hour. Sputums for acid-fast bacteria on three occasions gave negative results. The serum non-protein nitrogen rose to 60 mg. per cent. Within the week he expired at the age of thirty-seven. Reportedly death was due to "coronary occlusion and congestive heart failure." No autopsy was performed.

Our two patients, P. V. and E. V., have three other siblings. A sister, age thirty-eight, is asymptomatic and normal by physical examination; normal results were obtained from oral vitamin A and glucose tolerance tests. (Table 1.)

TABLE 1

Patient	Glucose Tolerance Test	Fasting		Six Hours After Oleum Percomorphum	
		Vit. A (μg. %)	Carotene (μg. %)	Vit. A (μg. %)	Carotene (μg. %)
E. V. (sister)	Normal	25.2	105	70	227
P. V. W. (sister)	Flat × 2 (rise 26 mg. %)	4.3	265	78	242
P. V. (daughter)	Normal	18.1	136	99	206
M. V. (daughter)	Normal	22.4	136	100	165

Another sister, age thirty-one, has had a two- to four-plus albuminuria persistently for at least eight years. At age eighteen there had been some pitting pretibial and ankle edema for about one month. For eight years there had been a chronic slightly productive cough, occasional asthmatic attacks, chronic fatigue, intermittent aching in the back of the legs, shoulder joints and under the left scapula, and intermittent abdominal cramps associated with gas. She has had temporal mandibular arthritis thought to be secondary to malocclusion. In 1947 there was a "toxic dermatitis" of the extensor surface of the forearms, shoulders and back. Physical examination revealed the subject to be normal except for a slight yellowish tint to the skin. The blood pressure was 118/78 mm. Hg. The twenty-four-hour urinary excretion of albumin was 5.1 gm. Hematologic studies, basal metabolic rates, stomach and small bowel x-ray studies, retrograde pyelograms and chest and joint x-rays gave normal results. The oral glucose tolerance curve was flattened and the fasting vitamin A level was 4.3 mg. per cent, the serum mucoproteins 2.8 mg. per cent, serum non-protein nitrogen 25 mg. per cent, albumin 4.2 and globulin 1.7 gm. per cent. The Kline exclusion test gave negative results. Stool fat content was within normal limits. There were no red cells and only an occasional cast in the

urine specimens. The urea clearance test shows 45 cc./minute standard and 54 cc./minute maximal clearance.

The remaining brother, age twenty-four, is a mongolian idiot and has been institutionalized since the age of eight. In 1943 he began to have

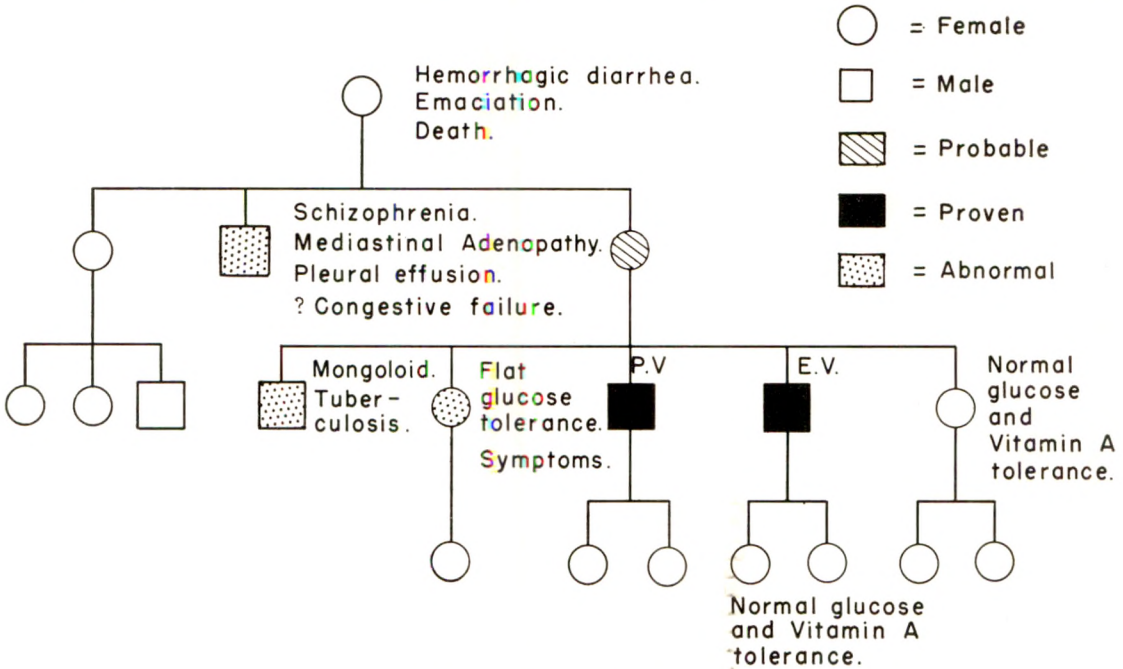


FIG. 11. Diagram of family history of patients P. V. and E. V. (Cases II and III).

temperature elevations, a rapid erythrocyte sedimentation rate and leukocytosis. Later in the year he developed bronchopneumonia in the mid-portion of the left lung. This cleared. In 1945 scarring at the apex of the right lung was noted. In 1950 the entire right lung and the upper two-thirds of the left lung showed small dense nodular shadows distributed evenly over the areas. Later there was a soft infiltrate noted in the apical areas and by 1953 x-ray examination revealed that the lesions had undergone considerable resolution although there was nodulation and fibrosis at both apices. It is assumed that he has tuberculosis although, due to lack of cooperation by the patient, this has not been bacteriologically confirmed. We have no information as to symptomatology or treatment received.

All seven children of the next generation are girls. The two children of patient E. V. gave normal reactions to vitamin A and oral glucose tolerance tests.

CASE IV. W. McC., a forty-nine year old white married factory worker, was first seen at the Henry Ford Hospital in December, 1953. He complained chiefly of intermittent looseness of the stools for four years. This condition had required hospitalization about twice yearly and usually ceased promptly with treatment. An attack lasted about five days with three to seven stools daily. Some upper abdominal distress occurred during attacks but no severe pain or cramps. There was never any constipation or any blood, pus or mucus in the stools. Anorexia occurred with the attacks and there was some difficulty with bloating and excessive gas during remission. The stools were light brown to cream in color, malodorous particularly at the time of diarrhea, and occasionally contained undigested food. Because of his complaints an exploratory laparotomy had been done three years previously and many enlarged mesenteric nodes had been noted. No biopsy had been done. The appendix was removed. Two years previously he had passed a right ureteral stone. On one occasion a paratyphoid organism was found in his stool. In 1953 cholecystectomy was performed and tissue from the mesentery was removed but a report of the tissue examination was not available to us. He had been anemic from the onset of his illness and had received transfusions totaling approximately 20 units on several occasions.

Following an attack of diarrhea and a temperature elevation to 101°F. he had received two units of blood two weeks prior to admission. Maximal weight had been 192 pounds ten years previously, and his normal weight over the last five years had been about 150 pounds. Weight upon admission was 135 pounds. For some time he had had mild night sweats and occasional edema in the left foot. He had been having nocturia three to four times, for ten years. Four years prior to admission he began to have migratory polyarthritis with painful, swollen joints, especially of the feet, knees and shoulders. He had had this intermittently until a year prior to admission. There had been an episode of jaundice at the age of twelve years.

The mother and father were living and were apparently in good health. A brother had died in 1931 after a two month illness which, according to the patient, was due to a ruptured appendix. No further details were available and a post-mortem examination had not been done.

Physical examination revealed a well developed, thin, white man with generalized pallor. The skin showed a diffuse tan especially marked on the neck, the sides of the face, the forearms and the hands, and in the year-old cholecystectomy scar. He was edentulate. The blood pressure was 90/50 mm. Hg. The abdomen was hyperresonant throughout except in the left lower quadrant. There was deep paravertebral fullness and generalized abdominal resistance. A generalized perifolliculitis was noted and was most marked over the lower abdomen, thighs and lower legs and in a predominantly extensor distribution. In the latter areas there were perifollicular hemorrhages. This cleared rapidly following administration of a vitamin B and C preparation parenterally.

Studies showed the hemoglobin to be 9.2 gm. per cent, red blood count 3.59 to 3.79 million/mm.^{3}, cells hypochromic and microcytic by smear; white blood count 7,400/mm.^{3}, neutrophils 69 per cent to 77 per cent, eosinophils 1 per cent to 2 per cent, monocytes 0 per cent to 4 per cent; lymphocytes 19 per cent to 21 per cent; sedimentation rate 26 mm./hour (Wintrobe), hematocrit 32 per cent, serum nonprotein nitrogen 30 mg. per cent, cholesterol 98 mg. per cent, prothrombin time 14 seconds (90 per cent), albumin 4.1 and globulin 2.0 gm. per cent, serum amylase 75 per cent and lipase 78 per cent of normal; vitamin C level 0.20 mg. per cent; sodium 138 mEq./L., chloride 104 mEq./L.; calcium 9.2 mg. per cent, and phosphorus 3.4 mg. per cent. Urine examinations including cystoscopic examination, intravenous pyelograms and twenty-four-hour urine collections for acid-fast bacilli, gave normal results. The cephalin cholesterol flocculation, the thymol turbidity and flocculation, and the BSP tests gave normal results. Agglutinations for typhoid, paratyphoid and brucellosis, and the Kline exclusion test gave normal results. The stools were liquid, contained bile and occasional undigested meat fibers, but contained no ova or parasites. The twenty-four-hour stool specimen contained 48 per cent fat on a normal diet and 14 per cent fat on a restricted fat diet. The oral glucose tolerance test in mg. per cent was 85 at 0, 97 at 15, 129 at 30, 154 at 90, and 130 at 150 minutes. Stool cultures consistently gave negative results. Proctoscopic examination revealed no abnormalities. A gastric analysis revealed free acid in all fractions except the fasting specimen. By biliary drainage the lipase was 47 per cent, trypsin 26 per cent and the amylase 28 per cent of normal, and all bile fractions were within normal limits. Barium enema showed spasm in the sigmoid colon but the postevacuation pattern was within normal limits. Stomach and small bowel series revealed a probable small hiatus hernia and a definite deficiency pattern throughout the small bowel.

While in the hospital the patient had an elevated temperature to 99.6 or 99.8°F. about every fifth or sixth day but was otherwise afebrile. A peripheral lymph node biopsy was done. He was treated on a full sprue regimen but, although he gained about 6 pounds, his diarrhea recurred and small bowel x-rays showed no change in pattern. As the lymph node biopsy showed evidence of Whipple's disease, he was started on steroid therapy. Enough time has not elapsed for evaluation of the result.

The lymph node biopsy (Fig. 10) showed a moderate proliferation of the reticulo-endothelial cells. There were occasional clumps of macrophages, especially in cortical areas, and these were strongly PAS-positive.

CLINICAL ASPECTS

The diagnosis of Whipple's disease should be considered in every case which exhibits the features of sprue and which is preceded by a migratory polyarthritis. The arthritis is manifested by pain, swelling, tenderness and increased heat and is indistinguishable clinically

from rheumatoid arthritis. Although not usually accompanied by residual deformity, the x-ray findings are occasionally compatible with those found in Marie-Strümpell disease. Actually the arthritis is the commonest symptomatic manifestation of polyserositis which on anatomic examination is found to affect in decreasing frequency the pericardium, the pleura, the peritoneum and the endocardium. Lesions involving mesenteric and retinal vessels have also been described. The arthritis sometimes disappears during attacks of diarrhea and almost always has subsided by the time the patient is presented for care of the sprue-like manifestations of the disease. However, the polyserositis may still be active as evidenced by persistent attacks of pleurisy, pericarditis, peritoneal irritation, fever, elevated sedimentation rate and, in some cases, leukocytosis and eosinophilia. The complaints are usually of abdominal pain, diarrhea, asthenia and weight loss, and the findings are most consistently those of emaciation and hypotension and may include lymphadenopathy, pigmentation or fever. Any combination of the laboratory findings common to non-tropical or secondary sprue may be present but a microcytic anemia is the most constant phenomenon. Low serum protein is also frequently present. Small bowel dysfunction may be demonstrated by flat vitamin A tolerance test x-ray deficiency pattern and often by a flat or delayed oral glucose tolerance curve. A doughy abdomen and/or an abdominal mass, usually on the left side, are helpful observations but apparently are not extremely common. To summarize, the over-all clinical picture is one which we would expect to see in the simultaneous occurrence of polyserositis and the malabsorption syndrome.

The clinical features of the disease can, however, be simulated by other entities and the only absolute diagnostic criterion at present is the typical microscopic findings in the small bowel and mesenteric lymph nodes. This includes the requirement that the macrophages be PAS-positive and essentially sudan-negative. Plummer et al.[22–23] have summarized the cases prior to 1953 and our references include all other cases reported to date.[1,4,8,10,15,24,26] As might be expected in a disease with such protean disturbances, cases have been published under the heading of Whipple's disease which, because of inadequate description of the lesions or because findings strongly suggest another entity, fail to satisfy this criterion. Ten cases have been indicted previously by others.[5,9,20] The case recently reported by Mendes[15] is characterized by atypical lesions; indeed, the diagnosis was rejected by reviewers of the material. Inguscio's case[10] completely lacks the study of tissue. However, we cannot agree with the opinion that the presence of chyloascites is reason to reject the diagnosis provided the other characteristic anatomic changes are present. There can be little doubt that lymph drainage eventually is blocked at the mesenteric nodes, that dilatation of the lymphatics takes place, and that eventually rupture and chyloascites could occur. Whether remission of the disease on non-specific therapy is reason to doubt the diagnosis cannot be stated emphatically. Certainly spontaneous remission and exacerbation is not uncommon in "rheumatic" disease or non-tropical sprue, the entities of which resemble this disease in many ways. It is similarly common to many diseases responding to cortisone and ACTH.

The disease is probably not as rare as the number of reported cases would seem to imply. Incognizance of the disease and the operative barrier to establishment of the diagnosis are probably the main factors responsible for the paucity of established cases. The material herein presented illustrates our own increasing awareness of the disorder, with diagnosis established twice by autopsy, the third time by laparotomy after the disease was suggested by peripheral node biopsy, and finally by peripheral node biopsy after the disease was suspected clinically. The latter means of diagnosis has obvious advantages and, because it avoids the hazards and expense of laparotomy and intestinal biopsy, can be undertaken more readily by patient and physician. This is apt to result in more frequent and earlier diagnosis. Since it is possible that the process is no longer reversible when in an advanced stage, which may account for the variable response to ACTH and corticosteroid therapy, early diagnosis may be of great importance.

Peripheral Node Diagnosis. Although peripheral lymphadenopathy is mentioned in about 50 per cent of the cases, few investigators have reported the results of microscopic examination. The presence of characteristic lesions in extra-abdominal nodes in this entity was first reported[14] in 1938. In 1952, in addition to reporting typical changes in the peripheral nodes, Upton[25] emphasized the wide distribution

of glycoprotein-containing macrophages in the periaortic and bronchial lymph nodes, in the periadrenal adipose tissue and in the liver. In the few remaining cases in which microscopic examination of peripheral nodes is mentioned it is stated that these nodes are not involved.

Our experience has shown that the diagnosis can be established with confidence by examination of peripheral nodes in these patients. All three patients in whom microscopic examination of peripheral nodes was done showed characteristic accumulations of Schiff-positive macrophages, especially in cortical areas. The changes are suggestive even in sections prepared by the routine hematoxylin-eosin technic. There is generally a moderate hyperplasia of reticulo-endothelial cells in the medullary sinusoids but in addition one can distinguish the unusual appearing nests of macrophages which are situated in the cortical areas. These nests are fairly discrete and are composed of cells which are slightly larger than the normal reticulum cells because of the accumulation of increased amounts of cytoplasm. The cytoplasm frequently shows fine vacuolization. In contrast to Upton's findings,[25] the lesions in our nodes showed no giant cell formation nor any similarity to a tubercle. Schiff stains disclose a marked reaction in these nests of macrophages and provide a pattern which we have not encountered in other processes.

In addition, our two autopsied cases confirmed completely the widespread nature of the distribution of the Schiff-positive material, as described by Upton.[25] Accumulations of macrophages were found in loose connective tissue of the retroperitoneal space and in focal accumulations in the liver. The generalized distribution of the material parallels the reticulo-endothelial system and suggests that phagocytosis by reticulo-endothelial cells is responsible for the material encountered in these areas.

Role of the Adrenal. The role of the adrenal gland in this disease is of interest and several observers[9,13,25] have reported the 17-ketosteroid excretion to be at the lower limits of or below normal. Adrenal hypocorticism would explain certain striking features of the disease, such as pigmentation, hypotension and asthenia. The emaciation, diarrhea and abdominal pain which are also usually present by the time the patient seeks treatment necessitate consideration of Addison's disease. Although it is possible that a relative adrenal insufficiency does exist, we believe adequate information is at hand to state that there is no intrinsic defect in the hypothalamic-pituitary-adrenal system.

The pretreatment 17-hydroxycorticoid excretion of patient E. V. was at the lower limits of normal. This was certainly not in keeping with the clinical impression of the severity of the stress. At that time the patient appeared critically ill. Twenty-five U.S.P. units of corticotropin administered intravenously over an eight-hour period resulted in an increase in 17-hydroxycorticoid excretion (Table II) and eosinopenia which were within normal limits. Marked symptomatic improvement followed and he remained afebrile for the next two days. Furthermore, long-term stimulation with corticotropin gel continued to produce high levels of 17-hydroxycorticoid excretion. Therefore, if relative adrenal hypocorticism does exist, it must result from failure of ACTH synthesis or release. Stress of the proper dimensions, however, produced an adequate adrenal response as suggested by temporary remissions precipitated by surgical intervention in two of our patients. This implies an intact ACTH-release mechanism. The eosinopenia in response to epinephrine, demonstrated by Jones et al. in

TABLE II

Date	Treatment	Dosage	Creatinine Excretion	17-hydroxysteroid Excretion (mg./24 hr. equiv. to Compound E)
9-12	None	.....	10.70	8.73
9-13	ACTH I.V. over 8 hr. period	25 U.	5.70*	14.1*
10-19	ACTH gel for 2 weeks	100 U each day	1,250	27.4
10-22	ACTH gel for 2 days; product of another company	100 U. each day	1,790	37.4
12-16	ACTH gel for 1 week	20 U. each day	1,208	15.1
2-13	Dihydrocorticosterone for 3 weeks	100 mg. each day	980	19
2-14	Dihydrocorticosterone	100 mg. each day	1,070	16.8
2-26	ACTH gel for 10 days	80 U. each day	780	26.1

* Denotes 8 hour collection period concurrent with administration of ACTH.

their patient, tends to support this assumption, although the validity of this test as a measure of pituitary-adrenal function is in doubt. The malabsorption state may lead to malnutrition with specific depletions which are adequate to account for the findings. Indeed, it seems extremely likely that the mechanisms producing these features in Whipple's disease are similar or identical to those operating in the malabsorption syndrome. In the latter condition no defect in the hypothalamic-pituitary-adrenal mechanism has been demonstrated to our knowledge, although a number of patients have benefited from corticotropin and corticosteroid therapy. These considerations and the failure of the induced adrenal hypercortical state to produce a universally favorable and sustained response in Whipple's disease make it unlikely that deficiency of adrenal corticoids plays any fundamental role in the etiology or fatal outcome of the disease.

Of further note was that the patient treated with corticosteroids seemed unusually resistant to the development of adrenal hypercorticism. On a 1 gm. salt diet with supplementary potassium there were no manifestations of hypercorticism despite a total of 2,400 mg. of cortisone, 7,000 mg. of hydrocortisone and 8,000 units of corticotropin gel in a six and one-half month period. Cortisone seemed to have no effect upon his illness. Hydrocortisone produced a temporary remission after an exacerbation during corticotropin therapy. As measured by urinary 17-hydroxycorticoid excretion, 20 physiologic units of corticotropin gel administered once daily, was essentially equivalent to 100 mg. of hydrocortisone per day. By the same measurement, 100 units of corticotropin gel daily produced twice the response of either of the foregoing. The poor response to cortisone when compared to corticotropin, reported by Plummer et al., may reflect not only differences in pharmacologic properties of the several corticoids but to some extent the inadequacy of the cortisone dosage.

Etiologic Considerations. A primary purpose of this report is to record the unusual familial occurrence of Whipple's disease. The condition described by Whipple has been considered a sporadically occurring entity of unknown cause, and most reports have neglected familial and environmental history. Hendrix and Black-Shaffer[9] emphasized that all their patients were long term residents of North Carolina. They considered the family histories of no significance in two cases; they did not record the family history in the remaining two. Whipple[28] reported that the family history was not contributory. Blumgart[3] found a family history of "gout" in his most acceptable case. The father of Gaertner's patient[7] suffered from rheumatism and heart failure. Paulley[19] noted that a sister of his patient had disseminated sclerosis. More recently, Story and Sagild[24] reported that their patient's sister suffered from diabetes and that two brothers and a daughter had died from cancer. In some ways this resembles the initial report we received concerning our patients' relatives. Gagne[8] mentions that other members of the family had cardiac troubles but does not specify further. Albertini's patient[1] suffered at the age of sixteen and twenty-two from a condition diagnosed as rheumatic fever. The remaining cases contain no definite statement concerning family history. No previous cases of familial occurrence of Whipple's disease are reported.

The family history in Cases II and III is presented in as much detail as is available so that the reader might make his own evaluation. The mode of death of the maternal grandmother is interesting—but a definite conclusion as to the nature of her illness is impossible. The family regarded the illness preceding death of the mother at the age of forty-nine as identical with that of the brothers and emphasized the symptoms, time relationships and mode of death. Despite the fact that a carcinoma of the cecum was suspected in her case, the authors feel that it is likely that she, too, had Whipple's disease. It seems probable that the institutionalized mongoloid brother has tuberculosis, although conclusive diagnosis will probably not be reached prior to death. A younger sister has suffered periodically from myalgia and right shoulder distress, recurrent abdominal pain, constant asthenia and a temporal mandibular arthritis thought to be secondary to malocclusion. She has had a chronic albuminuria for several years. She has also had a persistently flat oral glucose tolerance test and a low fasting vitamin A level. Her prognosis would seem uncertain. The institutionalized maternal uncle developed over a year's time a course characterized by pleural effusion, cardiac enlargement, mediastinal enlargement (probably secondary to adenopathy), increasing serum non-protein nitrogen, elevated erthrocyte sedimentation rate and low fasting blood sugar. He is reported to have died of

coronary occlusion and congestive failure at the age of thirty-seven. A paternal cousin has rheumatoid arthritis. The two daughters of E. V. and another sister have normal oral glucose tolerance and vitamin A tolerance tests.

This instance of proved Whipple's disease in brothers is, in view of the rarity of the disorder, not apt to be accidental. The family history serves to emphasize possible hereditary factors. However, exposure to the same infectious or toxic agent deserves some consideration. No exposure to a toxic agent is known but the family had known exposure to brucellosis and tuberculosis in cattle from which they drank unpasteurized milk. In this regard, tuberculosis with caseation was discovered in a mesenteric node and at the apex of one lung in the patient reported by Christie and Galton, and a caseous node is reported by Whipple.

A survey of the reported cases of Whipple's disease suggests in three ways that a genetic mechanism may be of etiologic significance: (1) The great majority of the cases have been in males. This is overwhelmingly true if challenged cases are eliminated. Although this might be explained by exposure to an agent not encountered by females (perhaps occupational) or by hormonal influences, a genetic factor the expressivity of which varied in the sexes (a not uncommon genetic condition) seems much more likely. (2) All cases thus far reported have occurred in the white race although there has been no special preference for specific nationalities. Lack of exposure to a disease agent encountered by the white race seems unlikely, especially in the United States. Racial immunity seems equally unlikely. The nationality of the patient has not always been stated but when recorded shows no preference. Cases have been reported from Scandinavia, the Continent, Italy, England and South and North America. There has been no specific mention of one of Jewish descent. This situation is strikingly similar to that encountered in non-tropical sprue. (3) All but two cases have occurred after the age of twenty-five. In one of these exceptions (Kampmeir, Case 2) the description of the microscopic pathology is weak and there is no information as to the nature of the material contained within the macrophages described. The other exception (Kampmeir, Case 3) is a thirteen year old girl with clinical and microscopic evidence establishing the diagnosis of lupus erythematosus and Whipple's disease. This consistency in age of the onset of the illness is worth noting since it is common for many hereditary diseases to manifest themselves over a selected age range.

The genetic factor therefore deserves consideration. In the light of presently known cases, the inheritance cannot be explained by a solely dominant, recessive or sexlinked characteristic. In regard to the latter, well established cases have been reported in women. If a genetic factor is involved, it would appear to be dominant with great variability in expressivity and penetrance. The inferred genetic relationship is very similar to that seen in the malabsorption syndrome. Like Whipple's disease, it is most frequently manifested in the white race without national preference, is frequently clinically latent for many years and has a higher incidence in adult life in the male.

Speculation as to the primary defect in this disease and its pathogenesis has been mentioned previously. Determination of several points might greatly clarify the situation. The first point is whether the intestinal absorption defect or the polyserositis is the primary condition. At the time when this might be determined the condition would not be recognizable clinically. Considering the possible hereditary nature of the disease, testing siblings and children of patients seems a logical approach. The secretory and absorption mechanisms of the gastrointestinal tract are the most reasonably tested. This was attempted, with the results previously recorded. More complete studies were performed only on the member showing some abnormality in screening tests and these studies failed to reveal further evidence of disease at this time. The second point concerns establishing the origin of the mucoprotein substance found in the macrophage, although it seems obvious from the distribution that the point of origin is in or through the intestinal mucosa. Of further aid in localizing suspicion to the epithelial cell of the intestinal mucosa is the observation by Frazer[6] of disordered metabolism of intestinal mucin in sprue-like states. The third point is the chemical nature of the mucoprotein and its relation to the increased serum mucoprotein demonstrated by Story and Sagild and confirmed by us. It is granted that some of the material must travel by way of the blood stream to reach the distant peripheral nodes. Were this not true, its presence in these nodes would infer a local origin. However, many other diseases manifest

high serum levels of mucoproteins. Many of these conditions have only inflammation and destruction of tissue in common, such as infarction, infections like pneumonia and meningitis, and malignancies.[17,27] Rheumatoid and Marie-Strümpell arthritis and gout show such elevations,[16] as does vitamin C deficiency in the rat.[21] None of these result in accumulation in macrophages in the distribution typical of Whipple's disease. It cannot at present be inferred that this increased serum mucoprotein bears any particular relationship to that seen within the macrophage.

From the standpoint of pathogenesis we concur in the opinion expressed in full or in part by several recent authors to the effect that the primary defect is probably a metabolic defect of the intestinal mucosal cell, resulting secondarily in a polyserositis as the result of hypersensitivity to a mucoprotein substance. This theory has several implications. Since all abnormal elevations of serum mucoproteins do not result in polyserositis, and since patients with Whipple's disease nearly all have some such manifestations, a difference in the nature of the mucoprotein or the "allergic" nature of the patient is inferred. Since the arthritis (or the pleurisy and pericarditis when symptomatic) is usually the first symptom, this implies a clinically "silent" intestinal defect existing frequently for years before it becomes symptomatic. This lends credence to the conjecture that it may exist from birth.

The possibility of a primary polyserositis triggering a latent intestinal defect, as infection frequently does in sprue, cannot specifically be denied although it has many shortcomings, not the least of which is the observed bowel changes.

SUMMARY

Four cases of Whipple's disease diagnosed between 1943 and 1954 are presented. Case histories of brothers proved to have the disease and extensive history suggesting possible abnormalities in other members of the family is presented.

Diagnosis by peripheral node biopsy is proposed.

It is concluded that pituitary-adrenal factors have no fundamental role in the etiology or fatal outcome of the disease despite the improvement, at least temporarily, of patients receiving ACTH or corticosteroid therapy.

The opinion is expressed that the disease is a hereditary one and transmission is not greatly different from that seen in the malabsorption syndrome. The primary defect is probably a metabolic one localized in the intestinal mucosa.

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Evidence for Serologic Hyper-reactivity in Sarcoidosis*

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VARIATIONS on the theme of the pathogenesis of sarcoidosis have been many but the theme itself has long remained one of host peculiarity. An aberrant individual response has been invoked by those favoring a relation to tuberculous infection[28] as well as more recently by those who have seen a histopathologic similarity between sarcoidosis and "allergic" tissue states such as periarteritis nodosa.[25]

The hypothesis that the host peculiarity might be an immunologic one has frequently been held. Cutaneous anergy to tuberculin,[6] the occasional finding of "anticutins,"[7,14] disturbances of the serum globulins,[29] relative insensitivity in sarcoidosis to BCG,[15] and the frequent modification of the clinical course of the disorder with steroid therapy[27] have all lent weight to this hypothesis. Very recently, two new pieces of evidence relevant to the immunologic aberration thesis have been presented. Friou[1] has demonstrated that patients with sarcoidosis are also less reactive cutaneously to oidiomycin, trichophytin and mumps virus; and Israel et al.[2] have shown the same situation to obtain with regard to histoplasmin.

In further exploration of immunologic considerations, Sones and Israel[3] found that in sarcoidosis there was neither an increased nor a diminished ability to make circulating antibodies to pertussis agglutinogen or typhoid vaccine. Since it has been previously shown by the mismatched blood technic that serologic hyper-reactivity exists in certain diseases in which hypersensitivity is thought to play a major pathogenetic role,[4,5] this method of estimating ability to make circulating antibodies was used to study serologic reactivity in sarcoidosis. The results obtained form the basis for this report.

METHOD

The individuals studied were of three groups: normal volunteers, patients with active pulmonary tuberculosis, and patients with sarcoidosis. The thirty volunteers were composed of presumed normal individuals who were either on duty at Fitzsimons Army Hospital or were awaiting return to duty following convalescence from orthopedic disorders. No other criterion was used in the selection of this group.

The twenty-one patients with active pulmonary tuberculosis were chosen from the tuberculosis wards of Fitzsimons Army Hospital and were tested shortly after admission. They were all bacteriologically proved cases. Fifteen had far advanced, five moderately advanced and one minimal disease. The nineteen cases of sarcoidosis were all proved by biopsy, and other causes of the sarcoid reaction such as histoplasmosis, coccidioidomycosis and berylliosis were ruled out as well as possible by history, cultures, skin tests and agglutination studies.

The normal group and the patients with tuberculosis were tested only on one occasion. The patients with sarcoidosis were given a six weeks' course of ACTH intravenously in the following dosage: 20 mg. over an eight-hour period each day for four weeks, 15 mg. over an eight-hour period each day for one week, and 10 mg. over an eight-hour period each day for one week. The patients were tested before

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therapy was begun and again between the end of the second week and the end of the fifth week of therapy. After ACTH administration had been stopped from one to three months, the patients were retested.

The testing consisted of the intravenous injection of 1 cc. of mismatched blood directly after obtaining a baseline serum sample for isoagglutinin titration. One week later another serum sample was obtained for isoagglutinin titer response to the stimulus of the mismatched blood. Titrations were performed in both saline and albumin diluents. The reasons for employing incompatible blood as the challenging antigen, the dosage used and the technic of titration are contained in a report published elsewhere.[4]

The titer responses of the group of patients with tuberculosis and the group of untreated patients with sarcoidosis were each compared with the titer responses of the group of normal volunteers by means of the "student t" test. This method assumes that the group of untreated patients with sarcoidosis, the group of tuberculous patients, and the normal group all came from the same population. This hypothesis then is evaluated by means of the formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{N_1\sigma_1^2 + N_2\sigma_2^2}{N_1 + N_2 - 2}\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

where: $\bar{X}$ = arithmetic means of values, σ = standard deviation, N = number of cases. Using a Probability (P) table for "t" with Degrees of Freedom (DF) equal to $N_1 + N_2 - 2$, a "P" value was obtained. A "P" value of 0.05 or less was considered to indicate a significant difference, and 0.01 or less a highly significant difference between the sets of data, thus refuting the hypothesis that all sets were from the same population.

In comparing the titer values for patients with sarcoidosis during and after ACTH administration with the values obtained prior to ACTH administration, each individual was used as his own control. The "student t" test was used, employing the formula:

$$t = \frac{\bar{d}}{\sqrt{\frac{\Sigma d^2 - \bar{d}^2 N}{N(N-1)}}}$$

where: d = arithmetic difference between the value before ACTH, and the values during and after such treatment, $\bar{d}$ = arithmetic mean, N = number of cases. A Probability table for "t" was used with Degrees of Freedom (DF) equal to N − 1. The significance of the "P" value was interpreted as already indicated.

TABLE I

CHANGE IN ISOAGGLUTININ TITER IN NORMAL INDIVIDUALS FOLLOWING INTRAVENOUS INJECTION OF 1 CC. OF INCOMPATIBLE BLOOD

Patient No.	Saline Titer		Albumin Titer		Fold-Increase	
	Before	1 Week	Before	1 Week	Saline	Albumin
1	56	112	7	14	2	2
2	448	224	112	224	−2	2
3	224	224	14	56	n.c.*	4
4	224	896	14	112	4	8
5	28	56	28	56	2	2
6	224	224	56	56	n.c.	n.c.
7	224	224	224	224	n.c.	n.c.
8	56	224	7	56	4	8
9	112	112	28	28	n.c.	n.c.
10	448	896	56	896	2	16
11	56	224	14	28	4	2
12	1792	1792	896	448	n.c.	−2
13	448	448	112	56	n.c.	−2
14	1792	1792	896	224	n.c.	−4
15	112	112	28	14	n.c.	−2
16	224	224	28	14	n.c.	−2
17	224	448	28	56	2	2
18	28	224	28	112	8	4
19	56	448	14	112	8	8
20	28	896	7	112	32	16
21	224	896	28	224	4	8
22	448	448	14	224	n.c.	16
23	896	448	28	56	−2	2
24	448	1792	56	448	4	8
25	448	448	224	896	n.c.	4
26†	3584	3584	1792	7168	n.c.	4
27	224	448	112	112	2	n.c.
28	224	448	28	112	2	4
29	448	448	112	448	n.c.	4
30	112	448	7	56	4	8

* No change: used in computation as a fold increase of one.
† Not included in computations for valid statistical reasons.

An attempt was made to relate the tissue changes in the biopsy specimens and the clinical symptoms and signs to the fold increases in saline isoagglutinin titer in untreated patients with sarcoidosis. This was done in the following manner: the relative numbers of giant and of epithelioid cells in the biopsy material were each graded zero to four plus, and the sum recorded for each patient. The same was done for relative amounts of fibrosis and of hyalinization in the biopsy specimens. The former elements were taken to indicate activity, and the latter elements were taken to indicate inactivity of the tissue changes. Next, each of a number of common symptoms and signs in sarcoidosis was graded zero to four plus and the sum recorded for each patient. The statistical method used in

TABLE II

CHANGE IN ISOAGGLUTININ TITER IN TUBERCULOUS PATIENTS FOLLOWING INTRAVENOUS INJECTION OF 1 CC. OF INCOMPATIBLE BLOOD

Patient No.	Saline Titer		Albumin Titer		Fold-Increase	
	Baseline	1 Week	Baseline	1 Week	Saline	Albumin
1	27	224	28	224	8	8
2	0*	14	0	7	4	2
3	56	224	14	224	4	16
4	224	448	56	224	2	4
5	1792	896	224	112	−2	−2
6	112	224	56	224	2	4
7	112	112	14	112	n.c.	8
8	896	896	112	448	n.c.	4
9	224	112	28	56	−2	2
10	224	224	56	56	n.c.	n.c.
11	224	112	28	56	−2	2
12	56	224	56	112	4	2
13	112	112	56	112	n.c.	2
14	896	896	224	224	n.c.	n.c.
15	448	896	28	1792	2	64
16	56	1792	0	896	32	256
17	896	896	224	448	n.c.	2
18	448	448	56	112	n.c.	2
19	1792	224	224	224	−8	n.c.
20	1792	1792	224	896	n.c.	4
21	448	896	56	56	2	n.c.

* When the baseline titer was zero and the response titer was 7 the fold-increase was assigned a value of 2; increments above 7 were assigned proportionate values.

correlating the clinical evaluations and the estimates of the tissue changes in the biopsy materials with the fold-increases in saline titer was to obtain a correlation coefficient (r) by the method of least squares. This approach is contained in the formula:

$$r = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{N}}{\sqrt{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{N}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{N}\right]}}$$

where: r = correlation coefficient, x = numerical expression for clinical evaluation or pathologic changes, y = fold-increase in saline titer, N = number of cases. The value of "r" may vary from plus one to minus one. A value of plus or minus one indicates complete correlation, while a value of zero indicates no correlation at all.

RESULTS

Table I lists the changes in isoagglutinin titer exhibited by thirty normal individuals receiving 1 cc. of mismatched blood intravenously. Table II provides analogous data for twenty-one tuberculous patients. Table III shows similar data for patients with sarcoidosis; nineteen of these patients were tested before ACTH

TABLE III

CHANGE IN ISOAGGLUTININ TITER IN PATIENTS WITH SARCOIDOSIS FOLLOWING INTRAVENOUS INJECTION OF 1 CC. OF INCOMPATIBLE BLOOD

Patient No.	Saline Titer		Albumin Titer		Fold-Increase	
	Before	1 Week	Before	1 Week	Saline	Albumin
1. Before ACTH Administration						
1	224	1792	28	1792	8	64
2	448	3584	112	448	8	4
3	448	3584	28	448	8	16
4	448	11,200	56	112	25	2
5	224	896	28	448	4	16
6	448	5600	224	896	12	4
7	1792	22,400	448	1792	12	4
8	448	3584	112	448	8	4
9	112	448	56	224	4	4
10	224	1792	14	224	8	16
11	56	896	28	224	16	8
12	112	448	14	448	4	32
13*	1792	1792	1792	14,336	n.c.	8
14	28	224	28	224	8	8
15	224	224	28	56	n.c.	2
16	224	1792	56	224	8	4
17	28	448	14	448	16	32
18	896	896	896	224	n.c.	−4
19	1792	7168	112	448	4	4
2. While Receiving ACTH						
1	448	448	224	896	n.c.	4
2	224	224	14	448	n.c.	32
3	448	1792	112	1792	4	16
4	448	3584	56	896	8	16
5	448	896	112	448	2	4
6	....†	.....	.....	.....	...	....
7	1792	1792	112	448	n.c.	4
8	....	.....	.....	.....	...	....
9	112	448	56	224	4	4
10	224	1792	112	112	8	n.c.
11	224	224	112	112	n.c.	n.c.
12	28	896	28	112	32	4
13	....	.....	.....	.....	...	....
14	112	896	56	224	8	4
15	28	224	7	28	8	4
16	....	.....	.....	.....	...	....
17	224	896	224	224	4	n.c.
18	112	448	28	112	4	4
19	....	.....	.....	.....	...	....
3. Following ACTH Administration						
1	....	.....	.....	.....	...	....
2	448	448	224	224	n.c.	n.c.
3	....	.....	.....	.....	...	....
4	448	896	56	224	2	4
5	448	448	56	112	n.c.	2
6	....	.....	.....	.....	...	....
7	....	.....	.....	.....	...	....
8	....	.....	.....	.....	...	....
9	....	.....	.....	.....	...	....
10	896	896	112	224	n.c.	2
11	224	112	14	28	−2	2
12	224	112	56	112	−2	2
13*	1792	14,326	112,000	7108	8	−16
14	224	224	28	224	n.c.	8
15	224	112	7	28	−2	4
16	1792	1792	112	448	n.c.	4
17	3584	448	3584	896	−8	−4
18	....	.....	.....	.....	...	....
19	....	.....	.....	.....	...	....

* Patient not included in computations owing to doubt as to technical and statistical validity.
† Test not performed.

administration, fourteen of the same group were tested again during steroid therapy, and eleven were tested after ACTH treatment. Nine patients were followed by testing at all three periods. Table IV is a statistical data summary chart. Here it can be seen that there is no therapy from the response before such treatment. But after steroid therapy titer response was significantly lower than it had been before therapy, as can be seen by the "P" values in Table IV. Treatment of statistics was difficult owing to the relatively few patients in these

TABLE IV

DATA SUMMARY CHART

	No. of Patients (N)	Saline				Albumin			
		Base	1 Week	Arithmetic Difference (A)	Fold-Increase	Base	1 Week	Arithmetic Difference (A)	Fold-Increase
Normal	29								
Average titer ($\bar{x}$)		354.3	538.8	184.4	3.17	110.6	188.8	78.2	4.14
Standard deviation (σ)		444.	495.	350.	5.98	227.	233.	270.	2.11
Patients with tuberculosis	21								
Average titer ($\bar{x}$)		535.	550.	15.3	2.71	82.7	311.	228.	6.4
Standard deviation (σ)		595.	530.	559.	7.96	84.6	422.	425.	14.1
* Probability (P)		0.25	>.9	0.25	0.7	0.6	0.2	0.15	0.4
Patients with sarcoidosis before ACTH	18								
Average titer ($\bar{x}$)		454.	3721.	3256.	8.72	127.	507.	380.	12.2
Standard deviation (σ)		531.	5500.	5090.	5.92	219.	502.	502.	16.25
* Probability (P)		0.5	<0.01	<0.01	<0.01	0.8	<0.01	<0.01	<0.01
Patients with sarcoidosis receiving ACTH	14								
Average titer ($\bar{x}$)		348.	1040.	692.	6.0	89.5	434.	344.	7.07
Average difference ($\bar{d}$)		−28.0	−2448.	−2420.	−2.64	−21.5	−74.	−36.5	−7.64
† Probability (P)		0.13	0.7	0.15	0.40	0.7	0.65	0.8	0.2
Patients with sarcoidosis after ACTH	11								
Average titer ($\bar{x}$)		851.	549.	−302.	−0.7	425.	252.	−173.	2.5
Average difference ($\bar{d}$)		650.	−1601.	−2251.	−10.5	387.	−33.6	−449.	−9.9
† Probability (P)		0.1	0.15	0.05	<.01	0.3	0.7	0.18	<.05

* Probability of chance difference from values obtained in normal individuals.

† Probability of chance difference from values obtained in patients with sarcoidosis before receiving ACTH.

significant probability that titer responses in the normal and tuberculous groups were different. In contrast, however, patients with sarcoidosis before steroid therapy exhibited saline isoagglutinin titer responses that differed from those of the normal individuals with a highly significant probability, whether judgment is based on absolute rise or fold-increase. Titers based on the use of albumin as the titration diluent showed the same highly significant difference.

There was no significant alteration in the isoagglutinin titer response during steroid categories and the considerable spread of the data; hence, it was believed appropriate to construct Figure 1. Here it can clearly be seen that titer response after steroid therapy was always lower than it had been before therapy.

Table V presents an attempt to give numerical values to the various pathologic changes in the biopsy specimens, and to the clinical symptoms and signs exhibited by patients with sarcoidosis. The degree of correlation between these factors and the pre-therapy fold increases in saline

isoagglutinin titer were then determined. It is obvious from the correlation coefficients in the right-hand column of Table v that no significant degree of correlation exists.

COMMENTS

Many of the previous studies on the nature of sarcoidosis may be regarded as immunologic in implication. Perhaps the first of these was the observation by Schaumann[6] that patients with sarcoidosis are apt to have a negative tuberculin skin test. This observation has been one of the most repeatedly and fully confirmed findings in the history of sarcoidosis.[7–12] Jadassohn[13] construed such lack of cutaneous reactivity into a positive concept of "Anergie," mediated by a specific immune process occurring in the course of tuberculosis. Martenstein[7] gave even

TABLE v

CORRELATIONS OF APPARENT PATHOLOGIC ACTIVITY AND INACTIVITY, CLINICAL ACTIVITY, AND FOLD-INCREASE IN SALINE ISOAGGLUTININ TITER, IN EIGHTEEN UNTREATED PATIENTS WITH SARCOIDOSIS

Patient No.	Giant and Epithelioid Cells*	Fibrosis and Hyalinization†	Symptoms and Signs of Clinical Activity‡	Fold-Increase in Saline Isoagglutinin Titer	Correlation Coefficient of:
1	4	3	10	8	Path. activity vs. path. inactivity: $\sim = .16$
2	3	0	7	8	
3	5	3	2	8	
4	1	8	7	25	Path. activity vs. reciprocal of path. inactivity: $\sim = .11$
5	4	2	6	4	
6	4	1	10	12	
7	7	2	7	12	Saline fold-increase vs. path. activity: $\sim = .22$
8	4	5	7	8	
9	5	4	9	4	
10	5	4	0	8	Saline fold-increase vs. path. inactivity: $\sim = .25$
11	3	1	2	16	
12	5	1	3	4	
13	2	0	5	8	Saline fold-increase vs. clinical activity: $\sim = .24$
14	6	7	7	1	
15	6	4	7	8	
16	6	7	5	16	Saline fold-increase vs. reciprocal clinical activity: $\sim = .107$
17	5	3	6	1	
18	4	4	0	4	

* The relative numbers of giant and of epithelioid cells in the biopsy specimen were each graded zero to four plus, and the sum entered in this column. This column was taken to indicate activity.

† The relative amounts of fibrosis and of hyalinization were each graded zero to four plus, and the sum recorded in this column. This column was taken to indicate inactivity.

‡ Each of a number of common signs and symptoms of sarcoidosis was graded zero to four plus and the total entered in this column. Above 10 was considered marked, 5–10 moderate, and below 5 mild clinical activity.

more exact expression to the concept of anergy by ascribing it to the production of specific anti-tuberculin antibodies which he termed "anticutins." The presence, but neither the specificity nor the role of anticutins in sarcoidosis, was confirmed by Pinner, Weiss and Cohen.[14] Further evidence of "anergy" was offered by Lemming[15] who noted that a negative Mantoux reaction persisted in patients with sarcoidosis who were vaccinated with BCG.

In addition to the peculiar behavior of the tuberculin skin test in sarcoidosis, another kind of aberrant tissue reaction has been reported. In 1941 Kveim[16] showed that ground-up node and spleen material from patients with sarcoidosis produced a nodular lesion when patients with

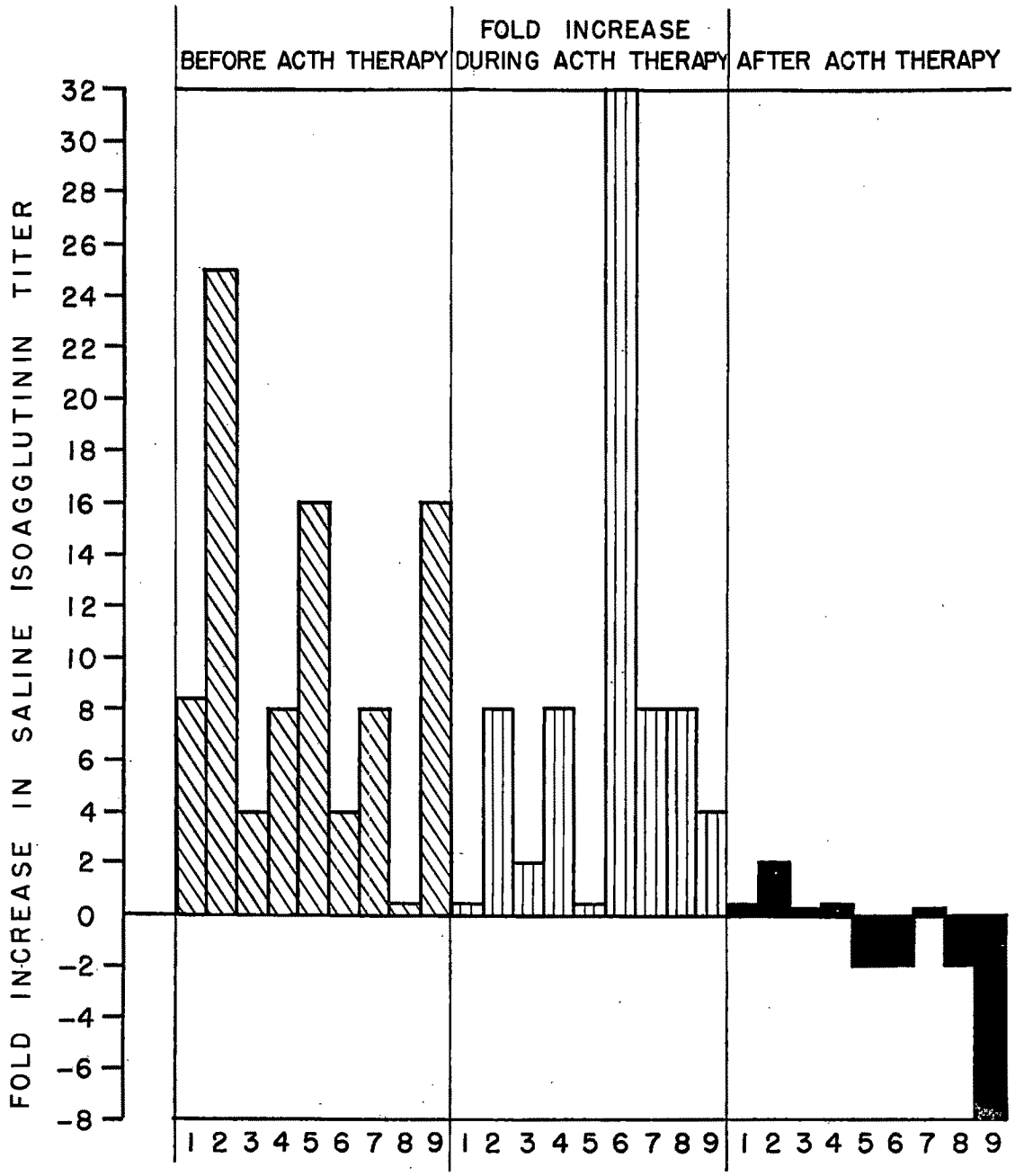


FIG. 1. Decrease in ability to manufacture isoagglutinins following steroid therapy in nine patients with sarcoidosis.

the same disease were inoculated intracutaneously with this material. This lesion, when examined by biopsy several months later, proved to be similar in appearance to those found in sarcoidosis. The finding suggested to Kveim a transferable agent as the cause of the disorder. This conclusion had to be amended following the work of Putkonen[17] and of Nelson[18] who showed that patients with sarcoidosis exhibited this reaction to certain non-sarcoid tissues also. The warranted conclusion has been retained, however, that the tissue responses in sarcoidosis are abnormal.

The essential distinction between inability of the skin to respond when all elements necessary for cutaneous reaction to tuberculin are present, and abnormality of one of the elements themselves was made by Urbach, Sones and Israel in 1952.[19] They found that when patients with sarcoidosis were inoculated intracutaneously with white cells from tuberculin-positive subjects and the site challenged with tuberculin, a positive skin test could be obtained. This was taken to indicate failure, in sarcoidosis, to manufacture or transport antibody rather than inability of the skin to respond.

The concept that the negative tuberculin reaction is in some way specifically related to the pathogenesis of sarcoidosis was finally vitiated by Friou's demonstration that cutaneous hyporeactivity also existed to oidiomycin, trichophytin and mumps skin antigen.[1] This observation was extended by Israel et al.[2] who found skin hyporeactivity to histoplasmin, and also by Sones and Israel who showed skin hyporeactivity to pertussis material.[3] These latter authors[3] also demonstrated a normal skin response in patients with sarcoidosis to intracutaneously administered histamine and to the Prausnitz-Kuestner reaction using ragweed pollen and appropriate serum.

Studies of serologic reactivity lagged behind studies of tissue reactivity. It was not until 1949 that Carnes and Raffel[20] demonstrated that complement-fixing antibodies to multiple antigens prepared from the tubercle bacillus were no different in level in patients with sarcoidosis than in normal, tuberculin-positive individuals. Similarly, Fleming, Runyon and Cummings[21] showed the same situation to obtain using the Dubos-Middlebrook hemagglutination test. At this point in the chronology of studies on sarcoidosis one could say that apparently tissue reactions and serologic reactions in the disease had different implications.

The two serologic studies described concerned static antibody levels obtained at a single point in time. A different approach, measuring antibody response over a period of time following a single antigenic challenge, was employed by Sones and Israel in 1954.[3] Using pertussis agglutinogen and typhoid vaccine they showed that patients with sarcoidosis manufactured circulating antibodies to the same extent as did normal individuals. Our finding of more than normal ability in this regard may be due to the particular antigenic challenge used in this study. The principal reason for employing mismatched blood in measuring antibody reponse in sarcoidosis was the previous experience that this technic demonstrated serologic hyper-reactivity in rheumatic fever and lupus erythematosus disseminatus.[4,5]

Our results indicate that serologic hyperreactivity is a frequent characteristic in sarcoidosis but not in tuberculosis. The kind of serologic behavior in sarcoidosis reported in this paper should be regarded as a characteristic of the disorder analogous to other descriptions, such as changes in serum protein patterns,[23] lack of caseation in the granulomatous lesions[12] or frequency of myocardial involvement.[12] The characteristic described does not in itself directly illuminate the question of the tuberculous etiology of sarcoidosis.

The attempt to correlate titer response, clinical status of the patient and pathologic changes in the biopsy material was unsuccessful. Three possible causes may be assigned for this failure: the members of the group were a non-homogeneous population; the method of evaluating clinical status and pathologic changes was fallacious; no correlation exists among the three elements. It is impossible to evaluate the relative roles of these factors.

The "P" values that express degree of significant difference among isoagglutinin responses of patients with sarcoidosis before, during and after ACTH therapy are listed in Table IV. It can be seen that the titer response after steroid therapy was significantly lower than before such therapy, although the titer response during therapy was insignificantly lower than the response before therapy. Moreover, Figure 1 reveals clearly that in every one of nine patients with sarcoidosis who was tested for saline isoagglutinin titer response before and after

ACTH therapy there was a lower fold-increase value after therapy than was obtained before. In fact, eight of the nine patients showed no fold-increase or a negative value on testing the titer response after ACTH administration. This is especially impressive if one considers that successively higher peak titers are to be expected upon repeated administrations of this antigen.[22–24] It seems a reasonable assumption, therefore, that ACTH was exhibiting its well known ability to curtail to some extent the ability to make antibodies[30] in these cases of sarcoidosis, although it must be admitted that we do not know if normal anamnestic responses are to be expected in this disease. It is tempting but wholly unwarranted to relate this diminution in ability to make antibodies to any therapeutic benefit the patients might have received from steroid therapy.

With the evidence for serologic hyper-reactivity presented in this report, observations on sarcoidosis fall into a strange dichotomy. On the one hand, a tissue abnormality seems to exist involving both a failure of response to certain intracutaneously administered agents and a particular kind of response to others (Kveim reaction); and on the other hand, there is increased ability to manufacture circulating antibodies to at least one challenging antigen. Certainly it is difficult to reconcile the idea of increased ability to make circulating antibodies upon intravenous antigenic stimulation, with hyporeactivity to cutaneous stimuli of an antigenic nature. And when one attempts to fit together increased ability to make circulating antibodies and a peculiar nodular tissue response to many agents one experiences a feeling of irrelevance if not indeed of conflict. One may force an analogy between, say, the serologic hyper-reactivity and particular tissue changes in sarcoidosis, and the serologic hyper-reactivity and particular tissue changes in lupus. But propriety and conviction both seem to be lacking in the analogy. Certain pathologic studies have been thought to suggest a similarity between the appearance and distribution of lesions in sarcoidosis and in periarteritis nodosa.[25] Again, the danger is one of forcing an analogy. Of course, certain aspects of sarcoidosis suggest a pathogenetic relation of the disorder to hypersensitivity. Eosinophilia,[12] disturbed serum protein patterns,[12] amelioration of the disorder when pregnancy supervenes[26] and clinical response to steroid therapy[27] are all circumstantial bits of evidence with a certain cumulative force. The finding presented in this report, of serologic hyper-reactivity to at least one antigenic challenge, is in consonance with the suggestion that hypersensitivity may play a role in the pathogenesis of sarcoidosis. But proof it is not. One must rest content with the observation that serologic hyper-reactivity is a frequent characteristic of sarcoidosis and await the clarifications that future exploitations of such observations may afford.

SUMMARY

1. Patients with sarcoidosis produce significantly higher titers of isoagglutinins in response to the intravenous administration of mismatched blood than do normal individuals. The response in patients with tuberculosis is not significantly different from that in normal subjects.

2. Attempts to correlate titer response with clinical status of the patient with sarcoidosis and nature of pathologic changes were unsuccessful.

3. Not only were anamnestic responses to the antigen not seen when patients with sarcoidosis were tested again after steroid therapy but also in every case the fold-increase in titer was less than it had been before therapy.

4. The significance of the finding of serologic hyper-reactivity is discussed in terms of possible relations of sarcoidosis to hypersensitivity.

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Review

Histoplasmosis*

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SINCE 1945, when Christie and Peterson[31d] and Palmer[146b] reported their remarkable findings concerning the frequency of positive skin reactions to histoplasmin, histoplasmosis has been recognized as a disease of considerable importance. It has been searched for and reported in many parts of the globe but has been clearly demonstrated to be endemic to a significant degree only in the eastern central United States.

It is true that, at present, the chief importance of histoplasmosis rests with the tendency to confuse it with other diseases, particularly pulmonary tuberculosis; however, the rapidly increasing number of correctly diagnosed cases calls for greater appreciation of the potentialities of this disease for morbidity and mortality in its own right. Criteria for the diagnosis of the disease have varied considerably; they should become more strict with increasing knowledge and availability of laboratory facilities. In the endemic area the diagnosis must be considered daily on the indirect evidence of skin sensitivity to histoplasmin in the presence of pulmonary (often calcific) roentgenographic aberrations and a negative tuberculin reaction. It is quite probable that histoplasmosis constitutes the most common cause of "fever of undetermined origin" in childhood in this part of the United States.

This paper represents an attempt to sift and organize the available information concerning histoplasmosis, to evaluate still existent problems and to report seven new proved cases illustrating the variability of the clinical picture.

HISTORY AND EPIDEMIOLOGY

The historical aspects of histoplasmosis have been covered in several readily available reviews[31a,31d,135b,187,188c,212] and will be only briefly recapitulated here. They are so closely related to the investigations of the epidemiology that it is proper and desirable to bring the two headings under joint consideration.

The first recognition of a previously unknown infecting organism was made by Darling in Panama in 1905.[42a] The patient, a twenty-seven year old Martiniquan Negro, had "pseudotubercles in the lungs" and focal necroses in the spleen, liver and lymph nodes at necropsy. In large mononuclear cells in these lesions and also free in the tissues, Darling observed round or oval bodies with refractive rims or capsules. Recognizing that these differed from Leishman-Donovan bodies for which he was searching, Darling suggested the name Histoplasma capsulata (sic) for the new agent. The name apparently was chosen to designate its tissue invasiveness, its resemblance to plasmodial protozoa and its refractive capsule. In 1908[42b] and again in 1909[42c] he reported similar observations in another Negro from Martinique and in a Chinese shopkeeper.

For almost two decades no further reports of the disease were made, and then a few scattered case reports appeared from Austria,[162] Minnesota,[202] Central America,[150] the Philippine Islands[201] and the Dutch East Indies.[137] As to the nature of the organism there was still some doubt, and the diagnoses were based upon the morphology of the parasite in infected tissues

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and on the resemblance to the pathologic data and illustrations in Darling's publications.

Although da Rocha-Lima,[43] from the study of slides of Darling's material, had suggested in a remarkable paper in 1912 the mycotic rather than protozoan character of the organism, it remained for DeMonbreun[47a] and Hansmann and Schenken,[76] independently in 1933, to identify conclusively the organism as a fungus and to describe its cultural characteristics. DeMonbreun's organism was obtained from the blood and tissues of a six-month infant whose clinical course was described by Dodd and Tompkins.[49] In the next twelve years case reports appeared with increasing frequency so that by 1945 Parsons and Zarafonetis[147b] were able to collect seventy-one cases, and shortly thereafter Iams et al.[91b] assembled a total of eighty-one cases. The disease was considered an almost consistently fatal, generalized infection with signs and symptoms depending upon the degree of regional or local involvement. In young children the clinical manifestations were regarded as fever, anemia, marked hepatosplenomegaly, diarrhea, failure to gain weight or weight loss, chronic cough and lymphadenopathy. In adults the features were looked upon as those of systemic infection, sometimes with predominant involvement of a single organ. Chronic granulomatous lesions or histiocytic proliferation in any body organ or system were thought to be the hall-mark of histoplasmosis.

A different concept of the disease arose from studies of non-tuberculous pulmonary calcification in roentgenograms. By the early 1940's it was becoming clear that the incidence of pulmonary calcification in roentgenograms of individuals who failed to react to tuberculin was higher in the east-central United States than elsewhere in the country.[31d,115,155] Noting that individuals from this region not infrequently reacted to relatively concentrated coccidioidin yet did not have coccidioidomycosis, Nelson and Furcolow (1940) speculated concerning other fungus infection as a cause for non-tuberculous pulmonary calcification;[188c] the skin sensitivity to coccidioidin would then be the result of cross reaction to an antigen common to two or more fungi. Smith[188b] confirmed the observations of Nelson and Furcolow and, reasoning from the geographic distribution of the fatal cases of histoplasmosis (the only kind then known), first suggested that H. capsulatum might be the organism responsible. The correctness of Smith's suggestion has received strong support from surveys of sensitivity to histoplasmin, an antigen prepared from cultures of H. capsulatum.[10,31b,146a,153b]

The incidence of positive reactions to this antigen is appreciably greater in the east-central part of the United States than in areas elsewhere in the country, and no other fungus antigen thus far tested approaches it in frequency of reaction. The obvious inference is that, as in coccidioidomycosis, there is a very widespread benign form of histoplasmosis much more common than the fatal form and that this benign form is responsible both for most non-tuberculous pulmonary calcifications and for sensitivity to histoplasmin.

Proof of the presence of mild or clinically silent forms of the disease has been slow in forthcoming. The evidence to 1950 was indirect and based largely on serial studies of pulmonary calcification in histoplasmin-sensitive individuals. Bunnell and Furcolow,[20] in 1948, in a report of ten proved cases of histoplasmosis included one boy of thirteen years as an example of mild illness with recovery. Proof was provided by the isolation of H. capsulatum from one of three gastric washings. The remaining cases were those of severe clinical illness with complete recovery, recovery probable or questionable and progressively fatal disease. Wheeler and his associates[204b] and others[13,108] have reported proved cases in moderately to severely ill children with symptoms and signs referable primarily to the respiratory system. Perhaps the best evidence is the report by Christie[31a] of the culture of H. capsulatum from lesions representing incidental observations in children dying from other causes or, at biopsy, of chronic cervical adenitis. These observations prove that inapparent infection does occur. Why similar observations are so seldom made in areas where the disease is believed to be endemic is by no means clear, unless it is simply a result of the failure to look for or demonstrate histoplasmosis.

Further support for the concept of benign forms of the disease comes from reports of laboratory personnel engaged in the handling of H. capsulatum whose skin sensitivity to histoplasmin changed from negative to positive.[68g] Seven of seventeen such persons in one laboratory had either clinical illness or lesions appearing in roentgenograms of their lungs. Organisms were not recovered from any of those affected. In Sweden[140] seven persons engaged in handling fungus cultures, including a strain of

H. capsulatum, became ill with respiratory symptoms and fever approximately two weeks after the first contact with H. capsulatum. Reactions in all seven subsequently were found to be histoplasmin-positive. Organisms were not recovered from any of the seven but skin tests on other members of the laboratory staff who had not become ill gave negative results, as did those in ten members from a medical department who presumably had had no contact with the organism. In two of the positive reactions, the results were confirmed by one of the authors (J. S.). The incidence of positive reactions is said to be only 0.6 per cent in eastern Sweden (Frisk, cited by Nilzen).[140] In this regard it is interesting that Christie[31a] states: "Among 44 patients in a tuberculosis sanitarium in Northern Sweden and in Swedish medical students, only one individual was positive to histoplasmin. He was born in Southern Rhodesia where histoplasmosis has been found."

An intriguing development has been the discovery[68f] of a possible relationship between infection with H. capsulatum and a series of "epidemics" of unknown etiology having many features in common.[60] Thirteen outbreaks are recognized, and several more are under study,[68a] although in seven of these "epidemics" six or fewer persons were involved. Two of the cases here reported (Cases I and II) constitute infections incurred in a fourteenth "epidemic."[1] Each epidemic was characterized by respiratory illness associated with disseminated pulmonary densities appearing in roentgenograms. Most of the epidemics were related to excavations or dust exposure in areas contaminated prominently by avian excreta or else remotely, if at all, used as human habitation. Attack rates were high and clinical and radiologic features were similar in the several episodes. In only two "epidemics" was the organism obtained from a patient during the acute phase;[1,116a,116b] in all others the diagnosis of histoplasmosis rests on the indirect evidence of positive skin sensitivity to histoplasmin, sometimes positive serologic responses, often the development of disseminated pulmonary calcification in roentgenograms and the isolation of H. capsulatum from soil in the exact area where the exposure is believed to have taken place. Isolation of the organism from soil was accomplished in eleven of the fourteen "epidemics."

This diagnosis in retrospect still suffers from the weakness of circumstantiality. Conversion from negative to positive reactions of skin tests is not assured in all those tested because pre-illness testing or testing early in the course of the disease was not done. The positive skin sensitivity found for years after the particular illness may have been present prior to it. In one epidemic in which skin test reactions were reported as negative during the acute phase of clinical illness, the incidence of positive reactions in the community in question is such as practically to preclude 100 per cent non-reactivity in any group of adults of the number involved.[170] Accepting the circumstantial evidence for H. capsulatum as the cause of this epidemic, one would necessarily assume that part of the group had exogenous reinfection. The mild cases may have represented some unknown reaction of such a group. Specificity and accuracy of serologic tests are still open to considerable question; the non-specificity of the roentgen shadows are well known. Even the isolation of H. capsulatum from the soil in the areas may indicate only that animals known to have natural infection with the organism had visited the site some time between the epidemic and the time the soil was studied.

Nevertheless, information is accumulating which indicates that the circumstantial evidence may well be correct. The organism has been found in soil in areas where the disease is prevalent[8b] and several different animal species have been found with natural infection in these areas.[129b,129c] The organism has been shown to be able to maintain a saprophytic life cycle in experimentally inoculated soil[58f] and may well do the same in nature. The relationship between rates of pulmonary calcification in roentgenograms and rates of histoplasmin sensitivity is so clear cut no one can deny that whatever causes the one must in large measure cause the other, and no organism other than H. capsulatum has been recovered consistently wherever the disease has been suspected.

GEOGRAPHIC DISTRIBUTION

Information concerning the geographic distribution of the disease comes from case reports and from surveys of sensitivity to histoplasmin.[133] Many reports are open to question so that in certain regions it is not yet proved that histoplasmosis is endemic. Nevertheless, it is safe to say that the disease is present in Africa, North, South and Central America, and possibly in some Asiatic islands and in Europe. Information from the Asiatic mainland and Australia is, at

present, inconclusive or else indicates absence of the disease. One case has been reported from New South Wales, Australia, diagnosis resting on histologic examination[95] of an excised skin nodule.

The area of greatest incidence is undoubtedly in the East Central United States. This includes the states of Missouri, Tennessee, Kansas, Kentucky, Illinois, Iowa, Ohio and Indiana. Sensitivity to histoplasmin falls off in all directions away from this contiguous cluster of states, and rates of sensitivity tend to vary inversely with the distance from this area. Unexplained local variations in the frequency of skin sensitivity occur even within narrow geographic limits of states, counties and cities.[68k,68l] Reports of cases and histoplasmin reactions are known in Canada[73d,74a,74b,191a] but the frequency is much less than in many areas of the United States. The southeastern provinces, those nearest the endemic area in the United States, are the ones from which the reports arise. No significant histoplasmin sensitivity has been found in Alaska.[183]

Mexico and the Central American countries show a relatively high histoplasmin sensitivity rate.[52a,84,181,198a,199b] Darling's three cases were found in Panama between 1905 and 1907, but none was in a native. No further reports of the disease there appeared for forty-five years[198b] except for one case of canine infection in 1945.[197] In 1951 a survey of 1,000 persons revealed 387 positive reactions to histoplasmin, of which 310 were also positive to tuberculin. Pulmonary calcification was found in roentgenograms of 113 persons.[198b]

In South America, too, relatively high frequency is noted but marked variations are observed from country to country and in different regions in the same country. Brazil[25] and Argentina both have areas of high incidence.[8,71b] A proved case has been reported from Chile[142b] where sensitivity rates thus far reported have been low.

The few reported surveys from India and China[133] indicate no significant incidence of histoplasmin sensitivity. A few reports from widely scattered areas in Africa show a low but definite incidence of histoplasmin sensitivity.[118]

In general, surveys from European countries, with the exception of Italy,[193] show very little or no histoplasmin sensitivity.[71a] Recent reports of clinical cases from France,[94] England,[40,48] Norway,[15] the Netherlands[177] and the Mediterranean countries[26,209] are based largely on circumstantial evidence of infection or else may easily represent imported cases from endemic areas.

It has been suggested by several investigators that areas of high incidence have in common a geologic situation of large rivers running through low countries.[164] However, there are numerous exceptions and, although humidity and dampness may be factors in perpetuation of the disease, other common features remain to be discovered before reasons for the geographic localization of H. capsulatum can be defined.[211b]

What is not known about the disease constitutes a challenge of considerable proportions for further investigators. It is not known whether there is any seasonal incidence. The reasons for the geographic distribution are unknown. Several[163,211b] have speculated concerning the effects of humidity, dampness of the soil, chemical composition of the soil, and so on. The portal of infection is not proved completely, although the evidence from a few well studied cases[110b,178a,178c] is very strong that, as in tuberculosis, it is largely respiratory. Rare primary lesions in the gastrointestinal system or in the skin or mucous membranes cannot be excluded and in some case reports appear to be probable. Transmission from human to human or from animals to humans has not been demonstrated; transmission from animal to animal (dog) has been suggested by studies of Prior and Cole.[153c] On the other hand, thirty-five male mice kept in plastic cages with infected females for two to six weeks gave negative reactions without exception (authors' unpublished data). The disease is so asymptomatic that man-to-man transmission may easily escape detection. From results of skin tests in cattle[68m,128,129f] it has been inferred that animals and humans in the endemic area obtain the infections from similar if not common sources. Studies of skin sensitivity among siblings[63b] suggest that factors outside home contacts are responsible for positive reactions in several members of a family.

HISTOPLASMOSIS IN ANIMALS

Histoplasmosis in dogs was first observed by DeMonbreun.[47b] Numerous instances in dogs and in different species have been found but the main point, whether or not there is any relationship between animal and human histoplasmosis, is still largely unanswered.

H. capsulatum has been demonstrated in cats,

mice, rats, skunks, foxes, opossums,[58a,c,d,e] bear,[39] raccoon,[129d] frequently in dogs,[34a,34b,128,129a,129b,129c,129e,153d] cattle[129b,129f] and possibly in a ferret[111b] and two horses.[157,162] H. capsulatum has been demonstrated in 44 per cent of fifty dogs and of fifty cats collected at random in Virginia.[169b]

Positive skin test reactions in animals are often obtained with concentrated or only slightly diluted antigen. Experimental infection seems to indicate certain specificity even of concentrated antigens; nevertheless one cannot fail to mention that, in general, with decreasing dilution the specificity of skin test antigens decreases. With this in mind, one can take notice of positive skin test reactions obtained in cattle, sheep, horses, swine, fowls, dogs and other animals.[34a,128,129c,129d]

With undiluted antigen, the skin tests of approximately eighty monkeys, maintained in a local research institution for periods of four months to three years, failed to elicit a single positive result. The animals had limited exposure to the endemic environment in an open "monkey island" in the local zoo during the summer months. The skin tests were performed on the upper lids of the animals (authors' unpublished data).

For obvious reasons wild animals cannot acquire the disease from man and are unlikely to be carriers directly responsible for human disease. It then becomes logical to assume that wild and domesticated animals acquire the disease from soil in a manner analogous to man; the cat, which comes in close contact with mice and rats, could possibly become infected from rodents.[58a]

Only the future will show whether the high incidence of positive skin test reactions in certain animal species really represents an expression of infection with H. capsulatum. In the first place, heterogeneous antigens and variable dilutions have been used in the different series, and comparison is hardly possible. Besides, it is well known that certain animals (mice, rats) hardly ever show positive skin test reactions while others react very strongly. It then becomes—until anatomic studies corroborate the skin testing—a matter of guessing whether, for instance, the high incidence of positive skin test reactions in horses (65 per cent)[129b] represents a true antigen-antibody reaction or whether other factors intervene.

Little has been published about the pathology of spontaneous histoplasmosis in animals[164] but excellent papers are available about cytology[16b] and other findings in experimental animals.[44,86e,106b,174] Monkeys[47b,210] golden hamsters,[53,159] mice,[47a,86e,106b,113a,147a] rats,[130] dogs,[61,129e] gerbils[16b] and guinea pigs[28,160] have been used in experimental work with uniformly positive reactions. In rabbits, however, results are equivocal.[28,77b,159] We found the mouse to be the animal of choice. Intravenous inoculation results in 100 per cent mortality, with an average life span of twenty-eight days in young male mice. Other authors favor intraperitoneal injections with or without mucin but results are never so constant or uniform as with intravenous inoculation. Schaefer[173] studied the influence of age on experimental histoplasmosis.

Intracerebral inoculation seldom produces lesions outside the brain.[101b] Brandt[16a] demonstrated the positive influence of x-ray radiation prior to inoculation on dissemination. Cortisone did not influence the course of experimentally inoculated disease in mice[9] but made dogs susceptible to infection.[61]

Interestingly enough, gross inspection of experimental animals from which H. capsulatum can be isolated often does not show any abnormality, one of the many intriguing diagnostic problems of the disease in animals. In three spontaneous cases in dogs a typical primary complex was found in the lungs and mediastinal lymph nodes. (Fig. 1.) While spleen and liver (Fig. 2) represent the best source for culturing from experimentally infected animals,[86c,106b] blood smears and cultures may sometimes be helpful. The former especially can give quick information about the number of circulating organisms. H. capsulatum has been isolated from urine of guinea pigs[159] and feces of dogs.[153c] As little as a single organism is sufficient to infect experimental animals (mouse).[3a]

Attempts to immunize mice have been reported recently;[171h,173] the immunity is relatively short lived.

SEROLOGY

The experience with serologic methods in the diagnosis of histoplasmosis has not advanced to a point where constant and unequivocal results can be anticipated. The reasons are as follows: (1) Too few verified active and progressive cases have been tested adequately to permit definitive conclusions; (2) several partially conflicting technics are in use which possibly reflect the



1 2

FIG. 1. Primary subpleural focus in spontaneous histoplasmosis of dog; central calcification; thin fibrous capsule; positive culture from satellite lymph node; original magnification × 28.

FIG. 2. Experimental (intravenous) infection; mouse liver; enormous swelling of phagocytes containing multiplying organisms; original magnification × 650.

response to different antibodies rather than to poor technic *per se*, and (3) very few laboratories do reliable, easily available tests.

The following technics have been described: complement fixation with an antigen from formalin-killed yeast cells,[171f] mycelial-phase broth filtrate (histoplasmin),[68d,171j,176,194] heat-killed whole cells[172c] and extract from ground yeast cells.[22e] Saslaw[172b] developed, in addition, a collodion agglutination test. While most observers agree that a positive serologic reaction with high titer may be found in active and progressive cases, contrary experiences have been recorded in which no antibodies could be detected in spite of progressive histoplasmosis[20,172a] or in spontaneously infected animals.[58e] Grayston questions whether prognostic significance can be attributed to the histoplasmin complement fixation test.[72a] Cross reactions, especially between histoplasmosis and blastomycosis[171b,d] and to lesser degree with coccidioidomycosis, have been observed both in naturally occurring disease and in sensitized experimental animals.[22b,c,58g,86b]

For practical purposes we believe that the presence of a positive complement fixation test at levels considered critical by the specific laboratory where the test is performed warrants further clinical and cultural investigation. The suspicion of active disease will certainly arise if the titer of the serologic reaction should be rising on serial tests. However, the importance of performing the tests on sera obtained at different periods of illness, if at all possible on one day, using identical conditions and antigen, cannot be overemphasized. We are at a loss to recommend one method in preference to any other; a negative serologic reaction, like any other negative reaction, does not rule out histoplasmosis.

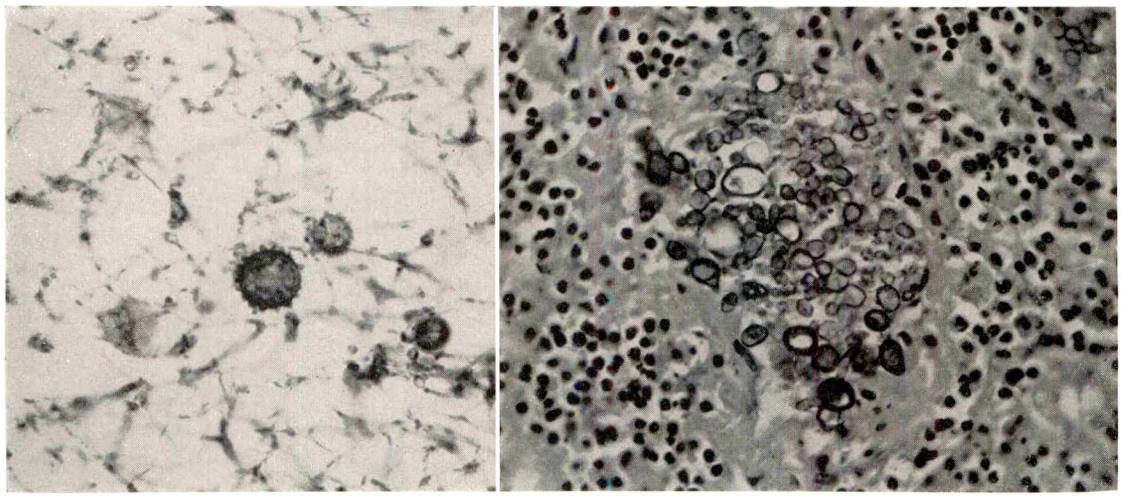
Cross reactions are common[171b,d] but can be easily detected if at least a blastomycin complement fixation test is run simultaneously. Whether or not a rising titer indicates dissemination and a falling titer improvement has not been established in a sufficient number of cases; exceptions are not uncommon. Attempts have been made to separate fractions of the antigen which would react with early or late cases only or which would not cross react, but this has been done only on a small number of sera.[22e]

Tests based upon the precipitin reaction[148,171i] and agglutination of sheep erythrocytes[142] have not passed into general use.

MYCOLOGY

Histoplasma capsulatum belongs to the Moniliacae of the Fungi Imperfecti[35] and is a biphasic fungus which, in tissue and on culture media kept at 37°C., appears as a small yeast (parasitic phase). At room temperature a mold develops (mycelial or saprophytic phase).[35,47a,86a]

The colony, after six to ten days on Sabouraud's dextrose agar, is small, white and cottony in appearance. The colony enlarges fairly fast



3 4

FIG. 3. Tuberculated spores at room temperature are pathognomonic for Histoplasma capsulatum. Small spores are seen elsewhere in the picture; original magnification × 1000.

FIG. 4. Case II; lymph node; giant forms in tissue explants. Compare size of organisms with lymphocytes. Original magnification × 650.

and becomes slowly tan to dark brown in back. A culture mount after two to three weeks (needed for the development of the tuberculate spores) reveals: (1) small spores which are sessile or on a short stalk and measure 1 to 5 μ.; these spores are smooth and spherical to pyriform; (2) larger round or ovoid smooth spores of 5 to 20 μ. often on short stalks; (3) tuberculate spherical to pear-shaped spores, which measure 10 to 25 μ. and are considered by modern authors to be aleuriospores.[58f,86a] (Fig. 3.) The latter are characteristic and resemble the medieval mace. The "projections" have been adequately described as thin points in the wall of the spores with cytoplasm pouring through the weakened spots in the wall.[51,86a]

The hyphae are generally thin. Septate and occasionally racquet mycelia are observed. The latter are found more often in cultures changing from the yeast phase to mycelial growth than in cultures kept constantly at room temperature. The number but not the diagnostic importance of the tuberculate spores has been overestimated in the past.[37,79] Certain strains from Georgia had very few tuberculate spores.[58e] Since particle size has been shown to be of importance in penetration into the alveoli of the lung,[18,19] it is indeed of interest to establish which spore size is prevalent.

Blood agar (found most efficient for growth from animal organs[86c]) can be used in addition to Sabouraud's medium, broth and corn meal agar for isolation of the fungus. Growth of the yeast phase is possible in liquid media; a small amount of agar (0.175 per cent) and a pH of 6.3 to 8.1 are essential.[171e] Even one drop of blood on the surface of Sabouraud's medium changes completely the growth character of the fungus which then appears flat, waxy gray-brown and without visible aerial mycelium.

H. capsulatum grows with ammonium as the sole nitrogen source and a sulfide or sulfhydryl group in a small organic molecule is essential for growth in the yeast phase. The presence of biotin is necessary for the yeast phase but has no effect on mycelial growth. Even at 37°C. change of phase can be accomplished by variation of culture media.[171g] The ideal pH is said to be between 7.2 to 7.6[38,86d] or 6.5 to 7.5.[86a]

On blood agar at 20°C. the appearance of the fungus is quite different from the appearance on Sabouraud's medium. The colony seldom becomes cottony, is gray, small, rather firm and leathery and sometimes has a slightly fluffy center. Tuberculate spores do not develop with the constancy which one expects on Sabouraud's medium. At incubator temperature the colony is slightly warty, dry-creamy or bacteria-like if growing profusely. Multiple budding of yeast cells has been described.[51]

Littman's oxgall agar does not support the growth of all strains and should not be used for primary isolation except in combination with the media already mentioned.

Conversion from the yeast phase to mycelial growth never fails but it is often difficult to convert mycelial growth with regularity to yeast without recourse to animal inoculation. Heavy inoculum, sealed blood agar slants (with or without cystine) and temperatures ranging from 34°C. to 37°C. are prerequisites for the change of phase, which may take as long as seven to ten days to develop.[125] Frequent subcultures (four to six days) are in general necessary to maintain growth in the yeast phase on solid media. Egg-yolk potato medium maintains yeast growth for long periods.[109,196] Once yeast growth has been induced in broth, maintenance is much easier, even if simultaneous mycelial growth may appear on top of the liquid. Rapid mass production on relatively simple media can be considerably enhanced by shaking action.[171c] H. capsulatum has been grown successfully on the chorio-allantoic membrane of the chick embryo.[103,135a]

An endotoxin has been described lately by Salvin.[171a]

The fungus is very resistant to physical exposure. It has been kept for four months in dried sterile soil, in water, ice (for over a year) and is reported as viable after heating at 62°C. to 63°C. for ten minutes[11b] or 45°C. for thirty minutes.[47a] The foregoing information would seem to suggest the necessity of public health measures to prevent burial of human or animal victims of the disease in view of the well founded belief that the soil constitutes a frequent source of infection. Certainly cremation would solve this problem.

The organism has been repeatedly seen in[58f] and recovered from soil by cultural or inoculation methods.[3b,58f,68i,72b,119,180a,211a] Lately it has been isolated from air in two areas which had given origin to outbreaks (epidemics) of histoplasmosis[92] and from an air sample in Iowa City.[168]

The morphology of the organism in culture mounts, in blood smear and in tissue is greatly influenced by the staining methods. With lactophenol cotton blue in the culture mounts, the fungus has partially stained cells; details about the sudanophil content are described by Howell.[86a] In the blood or bone marrow smear stained with the Wright stain or with similar stains, a pale bluish ring with focal chromatin generally is seen on one narrow side of the ovoid body and often the pigment is crescentic in shape. In tissue, with hematoxylin-eosin, small central dots surrounded by a pale halo are seen. Very seldom the ring form observed in blood smears can be reproduced in tissues with hematoxylin-eosin. If desired, a section stained with hematoxylin-eosin can be stained with periodic acid-Schiff stain without intermediate steps; the periodic acid decolorizes adequately.[103] With Schiff's reagent, using either the Hotchkiss-McManus or the Bauer stain, small red dots surrounded by a red thin ring or empty ring forms are visible. Occasionally, a disc-like figure a few micra in diameter can be seen representing the organism. Most authors agree that H. capsulatum has a capsule;[42c,58b,86a] a few deny it.[3c,103]

Budding is rarely seen in tissues or blood smears, in contrast to Blastomyces dermatitidis or Monilia which produce abundant budding forms in animal tissue. Larger forms have been observed occasionally in old blood kept in storage,[11b,67] and the organisms appear in general larger with the Hotchkiss-McManus stain[58f] because the whole cell is stained including the part which in hematoxylin-eosin stains seems to represent the capsule. Large forms of smooth outline have been produced lately in tissue explants from human and animal sources. Probably some factors producing tissue decay are connected with the development of the large spores.[180c] (Fig. 4.) Large forms have been seen repeatedly in Africa and a distinct species has been proposed by Vanbreuseghem as H. duboisii.[28,33,55,100a,b]

In retrospect, it is difficult to understand why culture of the organism was not accomplished until about thirty years after the discovery of the organism by Darling, since no special difficulties are encountered.

HISTOPLASMIN

The filtrate of cultures of H. capsulatum grown for several months in a synthetic broth[58g,86b] is used as antigen for skin testing. At present there is no purification of the antigen but standardization is attempted by titration in human beings or infected guinea pigs, taking Howell's original histoplasmin H-15 as standard.[185] Considering Howell's original 1–1,000 dilution of H-15 the "standard," it is possible to select single or pooled antigens which at a given dilution react similarly to the 1–1,000 dilution of H-15. Unfortunately, it is not made clear in many papers dealing with skin sensitivity studies whether given dilutions are comparable to the H-15 standards or are purely arbitrary. It would be imperative to have this

point clarified in the future in order to permit comparison of results obtained from antigens of different provenience.

The "ideal" antigen at critical dilution will give a minimum of heterologous skin reactions and an optimal number of homologous responses. The specificity may not apply to the same antigen when used for serologic reactions in the test tube. The question of specificity is indeed of prime importance. While there is no cross reaction with tuberculin, it has been shown both in men and experimental animals[58g,68l,86b,188a] that cross reactions do occur between histoplasmin and blastomycin, and to lesser degree also with coccidioidin and haplosporangin. Appropriate dilution of an antigen in general produces practical and useful results. Thus, for obvious reasons, the actual skin testing with histoplasmin should always include at least one other antigen, preferably blastomycin. In a recent survey it was shown[68l] that about a third of positive histoplasmin reactors also reacted to blastomycin and occasionally the reaction to the latter was larger in diameter than to histoplasmin. Experimental Candida albicans infection produced cross reaction to the histoplasmin skin test.[124]

Concentrated antigens are much less specific than diluted ones. Unfortunately, in certain animals (dog, cattle, horse) only relatively undiluted antigens (1:5, 1:10, 1:100) elicit skin reactions. In certain animal species (mice), skin reactions in experimentally infected animals can hardly ever be elicited whereas the guinea pig readily responds to intracutaneous testing.

In man the test is performed by intradermal injection of 0.1 cc. of antigen diluted to correspond to 1:1,000 of the original antigen of Howell (H-15). Any induration of 5 mm. and more is read as positive. Measurement of the induration and erythema is desirable. Erythema without induration is only rarely observed and should not be read as a positive reaction. Forty-eight hours after injection is usually accepted as reading time. A few reactions present at twenty-four hours disappear at forty-eight hours. On the other hand, true induration may become apparent after seventy-two hours or even later. In a survey, reading at forty-eight hours can be instituted arbitrarily and all other reactions can be neglected. In individual testing, it can become quite difficult to decide whether a transitory induration present at twenty-four hours and absent at forty-eight hours should be read as positive or not, and the same applies to reactions which appear after forty-eight hours.

It is generally assumed that a positive skin test in human beings represents a local demonstration of antibodies, indicating past or present infection. What is the evidence to support the assumption that a positive histoplasmin reaction means past or present infection?

1. It has been shown beyond any reasonable doubt that a positive reaction to the coccidioidin skin test with a dilution of 1:1,000, if given properly with appropriate antigen, signifies past or present infection with Coccidioides immitis. Since normal laboratory animals never show positive skin reaction to histoplasmin and since the development of a positive skin test can be observed after inoculation of Histoplasma, it seems permissible to consider the histoplasmin test as analogous to the coccidioidin test.

2. Many patients with proved histoplasmosis have a positive skin reaction to histoplasmin. By implication then, it is believed that a positive reaction occurs in persons who have or have had the disease.

3. Again as in the case of coccidioidomycosis, in which subclinical forms have been demonstrated to exist in apparently healthy individuals, it is suspected that asymptomatic histoplasmosis occurs. This belief is based on the prevalence of positive skin reactions in regions where clinical cases are most frequently observed, on the fact that animals in these areas have both positive skin reactions and clinical disease, and last but not least on the frequent observation of pulmonary calcification in tuberculin-negative persons with positive histoplasmin reaction.

Proof that subclinical forms of histoplasmosis exist is, at present, largely indirect. For completeness, it is necessary to enumerate the possible arguments against the conclusion that subclinical forms of histoplasmosis exist.

1. Incidental observations of asymptomatic histoplasmosis should be much more frequent at autopsy than actual experience seems to indicate. Whereas tuberculin-positive persons in the United States are decreasing regularly in number, occasional active and, more frequently, inactive foci are found at autopsy. The latter are thought to represent old, burned-out tuberculous lesions. Active foci of histoplasmosis have very seldom been observed as incidental findings, even in areas where the histoplasmin sensitivity rate is 70 per cent or greater. It is possible that an unknown number of calcific

pulmonary lesions which are classified as tuberculous are in fact due to histoplasmosis. Culture or histologic demonstration of H. capsulatum in calcified lymph nodes or burned-out lung lesions seldom has been accomplished (Ref. 31*a*, 58*g*, authors' Case VII) It is only fair to say that acid-fast bacilli are not demonstrated in every calcified focus. The possibility of such foci being of mycotic origin does exist, once more having in mind analogous findings in coccidioidomycosis[36,188c] and blastomycosis.[182d]

2. In only a relatively few of the proved cases of histoplasmosis have the patients had skin tests and not all have given positive reactions. Anergic reactions in severely ill patients are known in other diseases and may also occur here.

3. Large surveys have shown that a certain number of persons with calcified chest lesions do not react to any known antigen. From this finding we may conclude that calcified intrathoracic lesions occur independently in tuberculosis, histoplasmosis or coccidioidomycosis. It is only one step further to claim that all calcifications which are not due to tuberculosis are of unknown origin. Since a considerable number of persons with calcified lesions gave positive reactions, both to tuberculin and to histoplasmin, a specific answer in a given case may be beyond our present knowledge.

4. Clinical cases are rare or lacking[143] in surveys in areas where a high incidence of histoplasmin reactions exists, even where clinical cases have been found in men and animals.[153d]

5. The cross reactions may lessen the significance of skin or serologic testing. It has been shown that animals which are infected with Blastomyces may react strongly to blastomycin but, at the same time, to histoplasmin, coccidioidin or haplosporangin. This point must be considered in some detail.

Obviously, a positive reaction to different antigens brings several possibilities to mind. Either there is a common (partial) antigen in the four studied antigens (blastomycin, coccidioidin, haplosporangin, histoplasmin), or metabolic products of fungi are fairly similar in all four molds, or there may be an unknown factor which is responsible for the positive reactions. The probability of a common antigen is likely since no reaction can be elicited in healthy animals or healthy persons whereas in infected individuals cross reactions do occur.

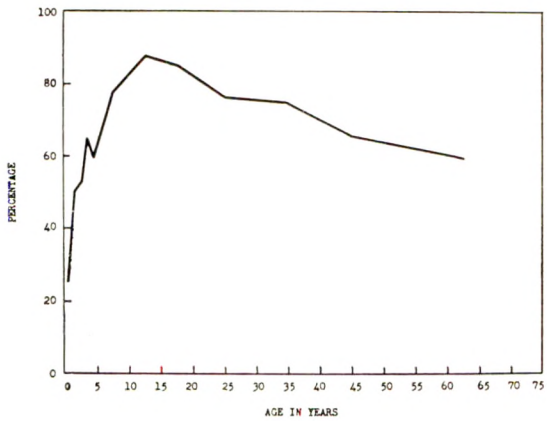
The fungi are not more antigenic than are their metabolic products. It has been demonstrated that addition of ground mycelia to the filtrate does not change the reaction or selectivity of the antigen. Obviously, heterogeneous organisms may have similar metabolic by-products and these metabolites serve as antigens. If there should be an unknown or neglected fungus which is responsible for the positive skin reactions in regions where calcified pulmonary lesions are common and tuberculosis may be excluded, this new fungus could possibly be responsible for skin reactions which now are read as positive for histoplasmosis.

Another observation which is very hard to explain is the decrease of positive reactors to histoplasmin in old age[211b] (Fig. 5) and the loss of reactivity which has been observed in specific individuals. It has become obvious that other skin reactions (tuberculin, coccidioidin) also become weaker or disappear with an increasing time interval between infection and testing. Why, however, in histoplasmosis the positivity of skin reactions decreases after forty years of age is not understood. Zeidberg,[211b] Furcolow[68e] and others have had considerable experience with retesting of individuals; alternating reversals of skin reactions have been observed even in proved cases. (Fig. 6.)

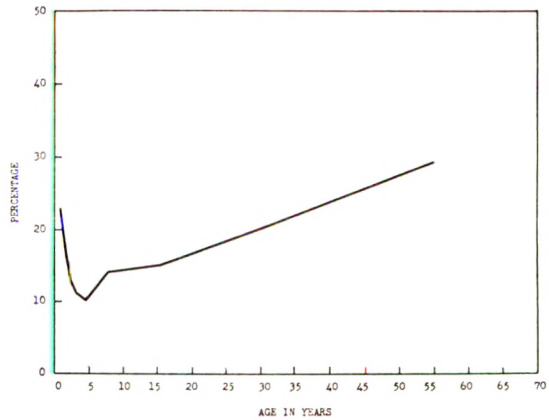
For practical diagnostic work, the importance of the histoplasmin skin test can be stated in the following way: A positive reaction to the best of our knowledge means past or present infection with H. capsulatum; a negative reaction does not rule out infection especially in severely sick patients.[200] There is still too little experience with the reaction in children of pre-school age to permit statements about the importance of negative reactions in this age group. Much of the discussion about the prevalence and distribution of histoplasmosis is based on results of skin testing. Lack of standardization of the antigen detracts from the reliability of certain results but in general it appears quite conclusive that: (1) High rates of skin reactors can be found mainly in Ohio, Iowa, Kentucky, Indiana, Kansas, Missouri, Tennessee and southern Illinois, and coincide with the geographic distribution of symptomatic cases. (2) Pulmonary calcification in the states indicated is more often associated with a positive histoplasmin reaction than with a positive tuberculin reaction. (3) Persons who gave negative results and were exposed to infection either naturally or in laboratories convert from a negative to a

positive skin reaction. (4) Certain infected animals convert, while to the best of our knowledge laboratory animals which had no contact with H. capsulatum never give a positive reaction.

For the sake of completeness it should be mentioned that a report of questionable significance has appeared with respect to positive reactions in penicillin-treated patients.[56]



5



6

FIG. 5. Typical frequency curve of histoplasmin sensitivity in relation to age. (From ZEIDBERG, L. D., DILLON, A. and GASS, R. S. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tennessee. *Am. J. Pub. Health*, 41: 80–88, 1951.)

FIG. 6. Incidence of reversal of skin test from positive to negative. (From ZEIDBERG, L. D., DILLON, A. and GASS, R. S. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tennessee. *Am. J. Pub. Health*, 41: 80–88, 1951.)

PATHOLOGY

The pathology of histoplasmosis is protean and, while a general pattern can often be recognized, no two cases follow precisely the same course. The parasite is usually found in phagocytic cells and for this reason, in generalized disease and in the experimental animal, liver, spleen, lymph nodes and bone marrow are filled with organisms.[2,103,151a]

Generally speaking, three reactions can be recognized in response to invasion of the organism by H. capsulatum. First, isolated organisms may be found in phagocytes with minimal or no tissue reaction of any form. Such observations are generally accidental or may be seen in lymph nodes which are at a considerable distance from the focus of inflammation. Frequently some nodes of the chain show more impressive reactions.

Second, often associated with a larger number of organisms marked histiocytic proliferation can be found which is most prominent in lymph nodes (Fig. 7), liver and spleen. Sometimes also whole alveoli in the lung can be seen filled with phagocytes containing numerous organisms. The sinusoids in lymph nodes in such cases may be completely obliterated, and beginning or progressive necrosis may be present.

Third, the granulomatous form can be observed. This occurs either in the productive (tubercle forming) or the exudative (caseating) variety (Fig. 8) and the two varieties may be seen simultaneously in the same organ or in different organs of the same individual.

Primary Pulmonary Complex. From observations in the literature and from our own experience in human and animal cases of histoplasmosis we conclude that the respiratory organs are the most common site of primary infection. In addition to the anatomic data, which will be discussed more extensively, there are also the epidemiologic facts which speak very much in favor of a respiratory portal of entry. We have in mind the epidemic outbreaks of the disease[60,68b,72c,116a,119] and the laboratory infections.[68g,140] In man, Schulz in two excellent papers[178a,178b] has described the appearance of primary pulmonary complexes which in every respect can be compared with tuberculous foci, that is, in size, shape and localization. Careful dissection and histologic examination of lungs from 105 consecutive autopsies in the endemic area revealed H. capsulatum in seventy cases with healed primary complexes.[191b] This figure

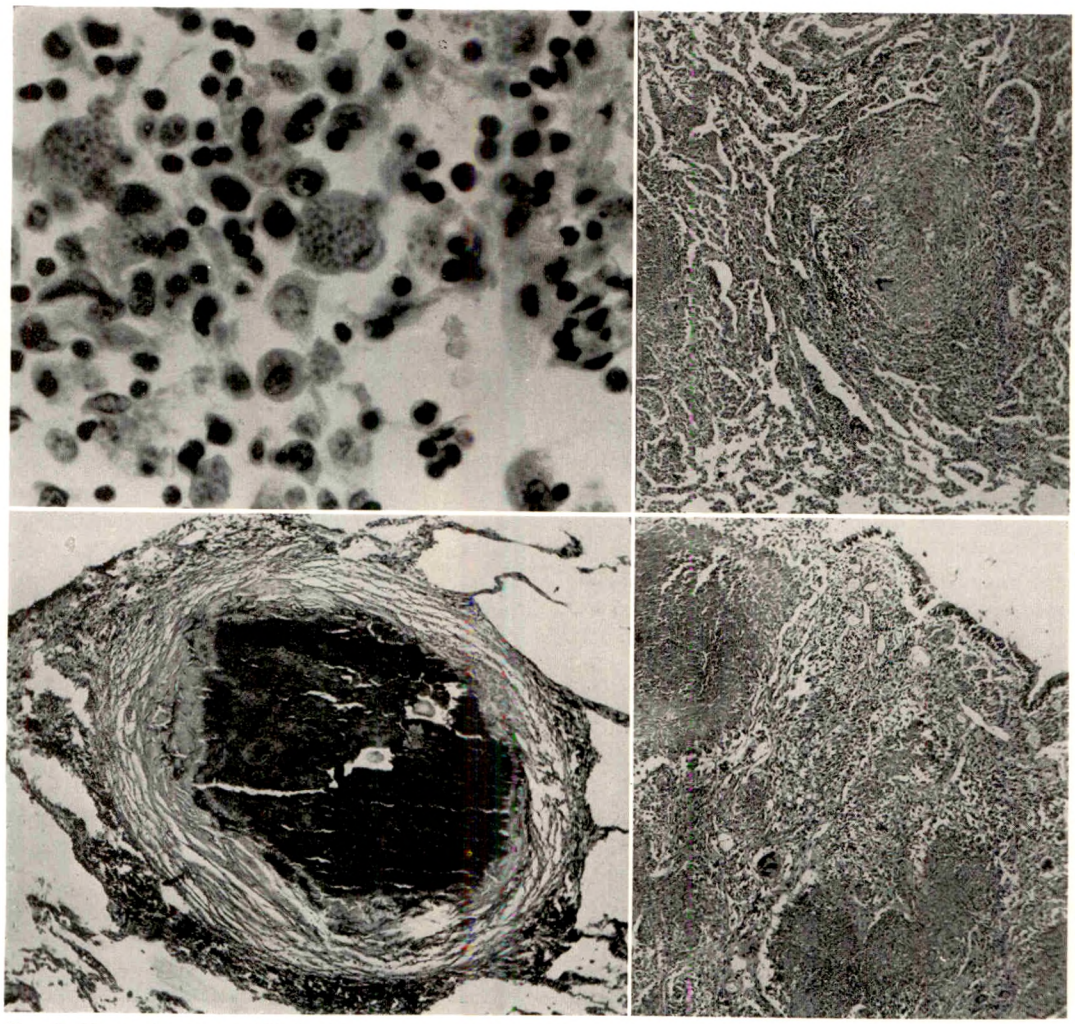


Fig. 7. Huge phagocytes filled with organisms in lymph node. (Courtesy Dr. M. L. Furcolow, Kansas City.) Original magnification × 900. Compare with Figure 2.

Fig. 8. Exudative and productive lesions in the same field; pulmonary histoplasmosis; original magnification × 65. (Courtesy Dr. M. L. Furcolow, Kansas City.)

Fig. 9. Calcified focus in a case of proved histoplasmosis; original magnification × 650. (Courtesy Dr. M. L. Furcolow, Kansas City.)

Fig. 10. Exudative caseating bronchopneumonia in histoplasmosis; original magnification × 65. (Courtesy Dr. M. L. Furcolow, Kansas City.)

corresponds with amazing accuracy to the incidence of positive reactions to histoplasmin in the same community.[68l] We have had opportunity to observe classic complexes in three human cases and in four dogs with spontaneous disease.[110b] The pulmonary focus presents itself as a rule in the form of a small, circumscribed, generally caseated subpleural lesion which is more frequently seen in the lower two-thirds of the pulmonary lobes than in the upper third. Frequently, the overlying pleura has a slight fibrinous reaction, or in older cases a small adhesion may form at this point. The regional lymph nodes are always hyperplastic but our experience has been that caseation is not so complete or universal in the regional lymph nodes as in tuberculosis. The lymph nodes are considerably enlarged and of rubbery consistency. Frequently the color is ochre and differs from the yellow caseation seen in tuberculosis. From x-ray examination and autopsy material we know that the pulmonary focus and the

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caseated lymph nodes can calcify. (Fig. 9.) How fast this occurs and whether it occurs regularly is not known. The multiple calcified foci so typically seen in histoplasmosis belong to a much later developmental stage. The cases with dissemination obviously produce massive invasion of the blood stream and hundreds of tubercules are formed in the interstitium of the lung; if these are necrotic, they later become calcified.[10,21a,b,31b,d,50,57,63a,68b,c,70,81a,b,120,123,145b,153a,168,187,216a,216b] Occasionally more than one primary focus can be formed. In tuberculosis a dozen or more primary foci may occur; it is likely that this occurs also in histoplasmosis.[7,20] Healing and calcification can be very slow and in some cases may not occur even after years.[63a,140]

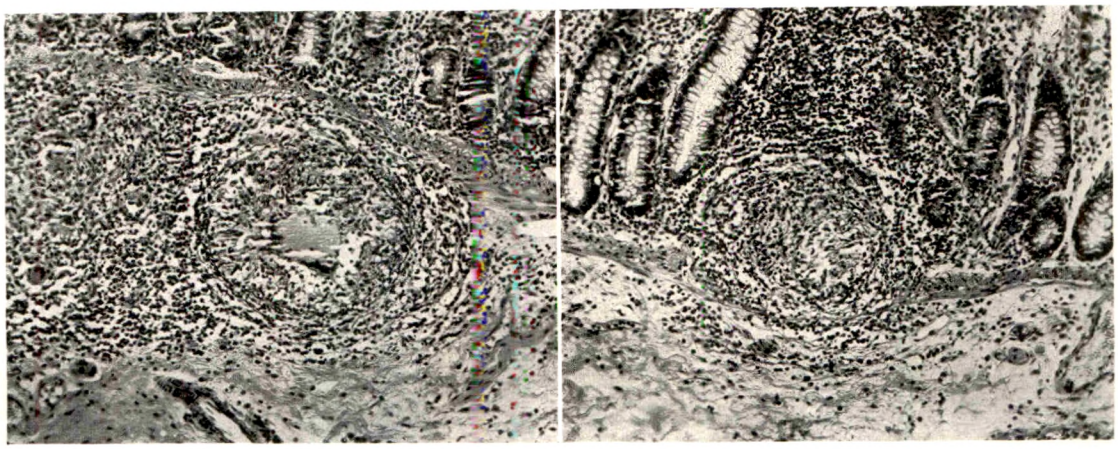
Evolution of Secondary Pulmonary Pathology. Lesions of histoplasmosis are seen in apical, subapical and other localizations; these may be coin-shaped and probably represent bronchogenic foci similar to the lesions observed in tuberculosis.[154,192] (Fig. 10.) The field of mycologic pathology is too young and too little material is available to permit more definitive statements. Whether these foci occasionally break down and are the source of cavity formation, or whether the cavities which are occasionally observed in histoplasmosis have a completely different origin is not known at present.[87,126,149] Occasional tumor-like (nodular) solitary lesions occur and offer an attractive field for surgery.[66,200,213] It is not known what the development of such solitary lesions would be if not treated. It is, however, well to remember that fungus diseases can have an extremely chronic course and the sequence of the developmental stages and allergic reactions is, at present, poorly understood.

While pleurisy may occur in any of the pneumonic involvements, it is seldom a predominant symptom clinically. In autopsy cases one can see the costal and visceral pleura studded with tubercles of a few millimeters in diameter, quite indistinguishable from the lesions observed in tuberculosis. Every known variety of exudate, including pus, has been found in the pleural cavity in selected cases of histoplasmosis. Scalene node biopsy has been successfully used in the diagnosis of histoplasmosis.[97]

Liver. The liver shows outstanding and very constant involvement in histoplasmosis, a fact which should be well considered for diagnostic reasons (liver biopsy)[4,13,180b] and for the prognostic outlook. The liver can become involved either by way of the hepatic artery or the portal vein, and, exceptionally (so far not reported), by umbilical veins or ascending from the duodenum via the bile duct (not reported). The most common source of infection of the liver, if there is bowel involvement, is probably through the portal vein. The hepatic phagocytes can become loaded to a degree which unquestionably must interfere not only with the specific functions of the reticulo-endothelial system in this organ but, by compression, also with the other functions of the liver. Sometimes we have seen sinusoids greatly distended and packed with swollen histiocytes; if the accumulation becomes very large, central necrosis may ensue. Less frequent than the histiocytic response is tubercle formation in the interstitium. One may find either very small foci of a few epitheloid cells with beginning central necrosis, or confluent or isolated well formed tubercles with occasional giant cells of the Langhans type. In overwhelming infection organisms may be found within liver cells or free in the capillaries and sinusoids. In addition to the more "specific" reaction there is frequently also cellular infiltrate, mostly of round cells, which sometimes can be quite prominent. Occasional histiocytes in the cellular exudate reveal the true nature of the inflammation.

Spleen. Splenomegaly, more often found in infants than in adults, is caused by hyperplasia of histiocytes which may be distended by numerous organisms. It is not unusual to see atrophy of the follicles and increase of the lymphocytes in the pulp. The capsule sometimes shows inflammatory reaction with fibrinous membranes which later become organized and fibrous. Tubercles, sometimes confluent and with caseation, are found in disseminated cases in combination with the histiocytic proliferation or as solitary lesions. One can only speculate why the spleen in infants is constantly enlarged, whereas in adults the enlargement is far less constant and less prominent. Since we do not yet know the role of reinfection, perhaps the answer will be found in the different pathogenetic development of disease in children as opposed to that in adults.

Adrenals. A rather specific and almost constant site of involvement is the adrenal glands.[200] A single organ or both organs may be attacked and any degree of involvement, from occasional small tubercles to massive and complete casea-



11 12

FIG. 11. Histoplasma "tubercle" in submucosa of large bowel, obviously of hematic origin; original magnification × 160. (Courtesy Dr. M. L. Furcolow, Kansas City.)

FIG. 12. Epithelioid cell nodule between mucosa and submucosa breaking through muscularis mucosa of large bowel; original magnification × 160. (Courtesy Dr. M. L. Furcolow, Kansas City.)

tion, is observed. The high incidence of involvement of the adrenals suggests some chemotactic mechanism. Histoplasmosis must be the cause of Addisonian symptomatology, especially in endemic areas where the prevalence of histoplasmosis infection is so much higher than tuberculosis in younger persons.

Intestine. Grossly the intestinal lesions are often very prominent both in number and in size. They represent either ulcerations of various sizes or infiltrates which can become quite polypoid and indurated. On the tips of such infiltrates small foci of necrosis or ulceration may develop. The regional lymph nodes can be found severely swollen and with foci of necrosis and even liquefaction. (Fig. 21.) On the other hand, intestinal lesions without marked regional lymphadenitis have been observed. Microscopically, one sees, as might be expected, enormous numbers of histiocytes loaded with innumerable organisms. Very frequently tuberculoid structures are formed but the location is often in the submucosa, indicating that at least two forms of intestinal involvement occur, that is, infection of the mucosa by deglutition and hematogenous spread to the submucosa with secondary breakdown of the tubercles toward the mucosa and formation of ulcers. (Figs. 11 and 12.) The lung is the main portal of entry but the occurrence of occasional primary intestinal infections cannot be denied, although they must be very exceptional. Again the similarity to tuberculosis, both in the pathogenesis and the morphologic character of the lesions, is obvious.

Bone Marrow. The bone marrow shows frequent involvement of a type which essentially follows the pattern described in the liver. Either there is a more or less diffuse proliferation of phagocytic elements which are distended by numerous organisms, or tubercle formation occurs.[152,166a,179] For further discussion, see the section on hematology.

Other Organs. Ulcerative lesions in the nasopharyngeal cavities are seen frequently in adults but are seldom observed in children. Perforating ulcers of the nasal septum have been described repeatedly and ulcerative lesions around the tonsils and even in the larynx are not unusual. It seems quite obvious that these lesions are not primary in nature since no regional lymph nodes are found, and it again becomes a matter of speculation as to why this localization should be so much more frequent in adults.

Any organ may be involved in generalized cases, including the heart, skin, central nervous system[49,178b] (Fig. 13), endocrine organs and bone (seldom).[46,54,101] References are given to papers dealing in somewhat more than casuistic form with localizations in specific organs or systems: mucocutaneous histoplasmosis;[41b,132,139b,145,195b] oral and nasopharyngeal involvement;[69,113b,135b,190,203,214] ocular;[44,107] adrenal disease;[158,195b,195b,200] splenic calcification in relation to skin sensitivity;[81,180e,216a,216b] gastrointestinal histoplasmosis and regional ileitis;[65,80,112,136,186,204a] heart;[11a,17,62] larynx[14,45,69,88,205] and urogenital disease.[41] Subendothelial proliferation has been recently described[2,11b,64] and the possibility of

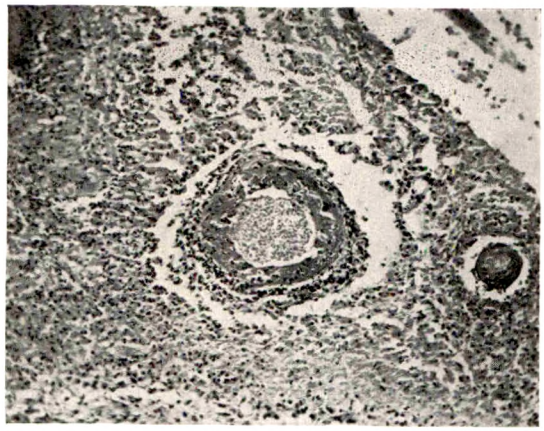


FIG. 13. Necrosis of vessel walls in meningitis in a patient with histoplasmosis. The morphology is practically identical with lesions seen in tuberculous meningitis and has all the implications of allergic reaction in reference to vascular necrosis. Original magnification × 160. (Courtesy Dr. M. L. Furcolow, Kansas City.)

focal necrosis secondary to vascular occlusion through bulging masses of subendothelial histiocytes is to be considered. Combinations with lymphomas[29,59,138] and sarcoid[23,93a,93b,151b,162] are discussed elsewhere and coincidental infections with B. dermatitidis,[111] C. neoformans[131,165,200] and M. tuberculosis[87,134,65] are on record.

HISTOPLASMOSIS AND LYMPHOMA

The coincidence of histoplasmosis with certain diseases of unknown etiology (lymphoma, sarcoid) has given rise to speculation whether the observations were purely accidental or a causal relationship existed. The combination of Hodgkin's disease[29,59,68c,63a,98,138,165] with histoplasmosis has been repeatedly reported. The authors had an opportunity to see slides from several of the cases and fully agree with the diagnosis based on morphologic grounds. Similar coincidental observations in leukemia have been reported.[110a,206]

Five main possibilities exist. (1) Coincidence: Statistically, two relatively rare diseases should not be found coexisting so frequently. (2) Malignant lymphoma predisposing to fungus infection: In view of the frequency with which other fungi (cryptococcus) also are found in malignant lymphoma, the possibility of predisposition due for instance to damage to the reticuloendothelial system by lymphoma, cannot be lightly dismissed. (3) Fungus infection predisposing to lymphoma: No evidence to support this view can be given at present. (4) The fungus actually causes histologic pictures similar or identical to what has been classified as lymphoma: No proof is available at this time. (5) Intensified search in lymphoma or sarcoid for organisms may give a higher number of positive reactions compared with more easily diagnosed tissue lesions; this is not very likely.

Very similar is the situation with respect to sarcoid where coexistence has been noted by competent observers.[93a,151b,160] Since at the present time the criteria for the diagnosis of lymphomas and sarcoid are mainly morphologic, the mutual relationship between these diseases and H. capsulatum is purely speculative.

HEMATOLOGY

In the numerous reports on the clinical features and laboratory findings in histoplasmosis, frequent mention is made of hematologic abnormalities that may occur to aid in the correct diagnosis. Chief among these is anemia, occurring either alone or in conjunction with leukopenia. The majority of the patients in whom hematologic data are available have had disseminated histoplasmosis so that knowledge of the alterations of the peripheral blood in the asymptomatic and non-fatal forms of the disease is relatively incomplete. Even in that form of disease which terminates fatally there is no "typical pattern" of hematologic change; normal results may be obtained[147b] and other hematologic entities simulated.[166b]

An attempt will be made to summarize the hematologic changes noted in different stages of infection with H. capsulatum as garnered from a review of reports in which sufficient data have been recorded. For details of observations made on patients reported up to January, 1945, the reader is referred to the review by Parsons and Zarafonetis[147b] in which blood counts are tabulated for approximately fifty patients. On the basis of this information and that obtained from material published since then[30,31c,59,62,64,83b,90,91a,91b,102,106a,122,138,141,144,152,165,166b,207] the following analysis has been made. Observations made on patients with proved histoplasmosis from the Children's Hospital are summarized in Table 1.

In this table representative blood counts are listed for those patients in whom multiple determinations were made. These were selected, insofar as was possible, before any form of therapy had been administered. Two patients exhibited rather marked fluctuations in total

white count. One of these is reported in detail herein (Case II, M. S.). In another patient (G. D. G.), previously reported by Christie,[31c] a diagnosis of visceral ascariasis or the Zuelzer-Apt syndrome might well have been suspected since at one time fever and hepatomegaly were present and a 32,150 white count with 36 per cent eosinophils was noted on differential smear. A liver biopsy at that time failed to reveal H. capsulatum but was also unlike the necrotizing granulomatous lesions seen in the Zuelzer-Apt syndrome. Six weeks later examination of the patient revealed the hematologic findings listed in Table I, at which time H. capsulatum was cultured from the marrow.

TABLE I

SUMMARY OF HEMATOLOGIC DATA ON ELEVEN CHILDREN WITH PROVED HISTOPLASMOSIS

Case	Age (yr.) and Sex	R.B.C. (mil./mm³)	Hbg. (gm. %)	V.P.R.C.* (ml./100 ml.)	Reticulocytes (%)	Platelets (per mm³)	W.B.C. (per mm³)	Differential				N.B.† per 100 W.B.C.	Marrow	
								P.M.N.	Lym.	Eos.	Mono.		Smear	Culture
C. S.	1, F	2.84	5.3	21	3.0	5,600	4,100	78	20	2	0	40	+	+
M. S.	4, F	3.94	8.2	28	2.2	230,000	9,100	71	23	1	5	0	−	+
B. S.	3, F	....	10.8	..	...	.....	14,400	..	..	..	..	..	−	+
B. J. P.‡	3½, F	2.9	7.9	..	2.7	80,000	13,100	75	14	..	11	0	+	+
G. D. G.‡	3 mo., M	4.14	8.8	33	...	80,000	6,100	29	53	..	18	3	−	+
M. C.§	2 mo., F	2.92	8.1	26	4.9	.....	4,700	41	47	..	12	2	−	+
R. P.§	11 mo., M	3.67	7.7	..	...	30,000	3,900	52	40	4	4	8	+	+
L. O.§	5 mo., F	2.91	6.1	..	3.6	110,000	1,200	58	42	..	..	1	+	¶
B. L. S.§	3 mo., F	2.72	7.1	..	...	.....	5,350	82	16	..	2	1	¶	¶
H. L.§	8 mo., M	3.8	8.1	..	...	110,000	2,150	52	38	8	2	16	−	¶
A. R. S.§	14, F	3.42	8.8	29	...	370,000	18,450	81	11	5	3	0	¶	¶

* V.P.R.C. indicates volume of packed red cells.
† N.B. indicates normoblasts.
‡ Previously reported by Christie.[31c]
§ Previously reported by Little.[114]
¶ Not done.

The pathogenesis of the hematologic changes noted is poorly understood. That there may be no single explanation for the frequently occurring anemia is obvious. Blood loss may be a factor in this disease, in which ulcerative intestinal lesions are found. Accelerated erythrocyte destruction, which occurs in various infections, may be an additional factor although there is no proof of its occurrence in histoplasmosis. Overt hemolysis seems likely, too, in view of the rapidity of recurrence of anemia in some patients despite transfusions and in the absence of obvious external blood loss. A microcytic hypochromic anemia in an infant found to have the disease may well be the result of preexistent nutritional deficiency of iron rather than a direct result of histoplasmosis itself. In certain instances the hematologic changes are identical with those seen in myelophthisic anemia and may then result from impaired function of the bone marrow organ secondary to overgrowth of parasitized reticuloendothelial cells. That the hematologic changes might also result from "secondary hypersplenism" or from suppression of bone marrow by a toxin liberated by the fungus is quite possible; however, there appears to be no satisfactory correlation between the size of the spleen and the degree of hematologic change, and the results of splenectomy have not been impressive.[5,144] Unfortunately, detailed hematologic studies in experimentally induced histoplasmosis in animals are lacking.

The anemia, which is usually of moderate severity but may be quite marked, occurs in approximately 75 per cent of patients with disseminated disease and in about one-third of those with local or non-disseminated histoplasmosis. Hemoglobin levels of 5 to 7 gm. per cent are rather commonly found in infants but are seldom so low in adults.

The size and hemoglobin concentration of red cells have not been sufficiently well studied to justify characterization of the anemia as normo-

cytic, normochromic, and so on. In two of the patients herein reported (C. S. and M. S.) the red cell indices indicated the presence of a microcytic, hypochromic anemia (mean corpuscular volume 71 and 74, mean corpuscular hemoglobin 18 and 21, mean corpuscular hemoglobin concentration 25 and 29). In one other (G. D. G.) previously reported[31c] but included in Table 1 the anemia was also microcytic and hypochromic. That this might occur often is not surprising in a disease which may be accompanied by ulcerative intestinal lesions and which has been reported rather frequently in infants and young children in whom iron deficiency anemia is so common.

Signs of active blood regeneration in the form of reticulocytosis, normoblastemia or alterations of red cell morphology are infrequently mentioned. A slight reticulocytosis (2 to 5 per cent) has been a frequent finding in children seen by the authors and has been recorded by others as well; in one instance it was as high as 48 per cent,[144] and this in an adult. Normoblasts in the peripheral blood would not seem to be a frequent finding in the cases reported, yet again from 1 to as many as 40 normoblasts/100 white cells have been noted in over half of our pediatric patients.

Polychromatophilia, aniso- and poikilocytosis and basophilic stippling have only occasionally been observed.

Leukopenia occurs at some time during disseminated histoplasmosis in from 50 to 60 per cent of patients. In half of these the white count is below 3,000, with counts as low as 800 reported.[31c] About one-third of those with nondisseminated forms of the disease develop leukopenia, which may be as severe as in widespread infections. There appears to be no correlation between leukopenia and either duration of illness or degree of splenomegaly. The leukopenia may be due to neutropenia but this is by no means invariable.

Leukocytosis is uncommon in the disseminated type of disease but occurs in at least one-third of those with clinical features which indicate localization of the infection to a single organ or organ system, for example, tongue, pulmonary system and so on. It appears to occur somewhat more often when lymphadenopathy is prominent. Leukemoid reactions may occur and marked fluctuations in the white count may develop from day to day (Case II, M. S.).

The differential white cell count is subject to wide variation and certainly exhibits no typical pattern. The percentage of granulocytes varies from very low to very high levels and there may be a distinct "shift to the left." Toxic granulation and other qualitative changes in the neutrophils have occasionally been noted. In a few instances monocytosis has developed. Eosinophilia is not prominent but isolated instances[90] of a marked increase in the percentage of eosinophils are recorded, as previously mentioned.

While histoplasmosis was first diagnosed during life by finding the fungus in circulating monocytes,[49] the organisms have since been found in peripheral blood smears in less than a dozen reported instances and then usually when the patient is in the terminal stages of the disease. Parasites may be found in neutrophils as well as monocytes. Kotcher[106a] called attention to the fact that demonstration of the fungi in peripheral blood is aided by making smears from the buffy coat. In two of the new cases reported herein, histoplasma were found in routine preparations of the peripheral blood in Case I and in stained smears of the buffy coat in Case III. It was more common to find a single organism engulfed but 3 to 4 fungi were often encountered and as many as 17 per neutrophils were found on one occasion. No special staining technics are required; routine Wright stain has been satisfactory in our work.

There are no studies available that help to clarify the etiology of the hemorrhagic tendency which has occasionally been encountered in disseminated histoplasmosis.[106a,114] Thrombocytopenia might, of course, contribute but has been recorded infrequently. Apparently, though, platelet counts have seldom been done. A moderate to marked thrombocytopenia occurred in almost half of our pediatric patients. (Table I.) One of the patients (Case R. P.) previously reported by Little[114] exhibited a marked tendency to hemorrhage in the terminal stages and was noted to have incoagulable blood in addition to thrombocytopenia and prolonged plasma prothrombin time. Another patient, herein reported (Case I), bled profusely for several hours prior to death. A marked thrombocytopenia was found but other studies designed to elucidate the defective homeostasis were not carried out. It is quite likely that the hemostatic defect is complex, such as occurs in parenchymal disease of the liver. The total serum protein in the patient R. P. referred to was in fact only 2.9 gm./100 cc.

Just as careful study of the peripheral blood may provide a clue to the correct diagnosis of histoplasmosis, morphologic study of the bone marrow may be diagnostic and its value definitely enhanced by simultaneous culture. Thus in one of the patients herein reported (M. S.) who was known to have widely disseminated disease, thorough examination of marrow smears at intervals during a protracted illness failed to reveal the fungi while marrow cultures for the organism almost invariably gave positive results. Of the nine patients in whom marrow examinations were performed (Table 1), histoplasma were found in the smears of four. In an additional four patients the culture gave positive results while the smear gave negative results.

There is a difference of opinion regarding the value of routine bone marrow cultures for H. capsulatum.[166a,179] Our experience would lead us to agree with Rohn[166a] that culture is of very definite value, especially in children. It was the single procedure establishing the diagnosis in a patient whose symptoms were minimal and not suspicious for histoplasmosis (Case v).

Detailed studies of the bone marrow in histoplasmosis, with differential cell counts, have not been reported. Usually one finds only statements regarding the presence or absence of organisms. Otherwise only gross estimates of cytologic changes are noted. Specimens are often said to be hypercellular and there may be increased numbers of macrophages; the latter may contain the fungi. As in the peripheral blood, however, H. capsulatum may be found in monocytes, neutrophils or plasma cells[166a] or may occur free in the marrow. There may be myeloid or erythroid hyperplasia and the number of plasma cells may be increased. Megaloblasts have been noted by two authors.[152,215] One adult patient may have had pernicious anemia.[152] However, the finding of megaloblasts in the bone marrow of infants with histoplasmosis, such as occurs in other disease states, is understandable and was in fact the case in two of the new patients reported herein (C. S. and M. S.). In one of these (M. S.) no reticulocytosis or alteration of the morphology of the marrow following folic acid therapy was noted. The morphology of the megakaryocytes had been inadequately studied. Provided parasites are not found, there seems to be nothing distinctive about the bone marrow in histoplasmosis.

Granted that there are obvious deficiencies in our knowledge of hematologic changes occurring in infections with H. capsulatum, it may be concluded that in an endemic region and in a patient with fever, respiratory symptoms and hepatosplenomegaly, the occurrence of anemia and leukopenia with or without thrombocytopenia is strongly suggestive of disseminated histoplasmosis. The presence of leukocytosis, however, should not deter one from suspecting the disease. Careful examination of the buffy coat from peripheral blood and of marrow may be diagnostic, and culture of the marrow and blood can certainly be recommended.

SEX INCIDENCE

There is a significant sex difference of proved disease in adults, males outnumbering females about four to one.[178a] In children the frequency is approximately equal in the two sexes. A decrease in the incidence of skin sensitivity to histoplasmin has been noted in females at the end of the second decade.[68h] The difference between sexes has been considered a possible expression of social patterns which limit exposure to infection but biologic factors cannot be excluded. No significant racial differences have been recognized.

CLINICAL FEATURES

The clinical manifestations of histoplasmosis can be derived from the many reports now available. Mochi and Edwards[133] in 1951 were able to assemble 370 papers dealing with clinical reports of histoplasmosis and with results of histoplasmin skin testing in various parts of the world. Proved cases have been described with signs and symptoms related to practically every body organ and system. However, when many of the reports are reviewed critically assurance that the disease causing the signs and symptoms is indeed histoplasmosis is often lacking. Diagnoses based on positive skin sensitivity, on roentgen observation, on the more recently developed immunologic procedures and even on microscopic studies of tissues removed at biopsy or necropsy can in many instances be discarded as inconclusive or grossly erroneous. This does not mean that the technics are valueless or that cultural technics alone are satisfactory; it does mean that every available method of diagnosis should be exploited in cases in which the disease is suspected and that the

criteria for suspecting the disease should be derived from those reports which withstand critical scrutiny.

It is generally agreed that there is a clinical disease spectrum of infection with H. capsulatum which extends from no apparent illness to fulminating fatal disease. For purposes of discussion, three broad classifications can be considered to bridge this spectrum. Considerable overlapping occurs in successive portions of the classification.

1. Fatal, Symptomatic Histoplasmosis. In children the fatal form of the disseminated disease is commonly characterized by fever, anemia, weakness and hepatosplenomegaly. The review by Iams and associates[91b] describes the clinical manifestations in great detail. The signs and symptoms depend in part on the degree of systemic involvement, in part on the extent to which individual organs are affected. "Toxicity" *per se* does not adequately account for fever which has been practically a universal observation. Widespread infection has been noted in children with little or no temperature elevation. It appears likely that the febrile reaction represents a response to secondary infection by bacteria capable of more pyrogenic activity than the fungus. Absorption of "toxic" breakdown products from necrotized parasitized cells may be related to the fever in the absence of secondary infection. No characteristic type of febrile reaction has been noted; hyperpyrexia is a not infrequent terminal event after a progressive septic type of fever. Prolonged or increasing pyrexia is an ominous sign.

Anemia of moderate to severe degree is usually present. The number of white blood cells in the peripheral blood tends to be depressed although occasionally leukocytosis is observed. A leukemoid reaction has been observed on many occasions and clinical and hematologic confusion with leukemia has been noted. Careful evaluation of young children or infants considered to have leukemia, who give a history of having siblings who died of similar complaints, may be very productive in the discovery of additional cases of disseminated histoplasmosis. Further discussion of the hematologic aspects of histoplasmosis is included in the section on hematology.

In adults, particularly the very old, the clinical course is not dissimilar. Frequently, histoplasmosis occurs as a complication of other serious, more or less chronic diseases. The association with Hodgkin's disease and sarcoid is commented on elsewhere in this paper. Pulmonary manifestations are more common in adults, as are localized ulcers of the mucous membranes of the upper alimentary or respiratory tracts. The course is like that of an overwhelming septicemia with rapid progression. A variant seen in adults is the chronic progressive form. This may persist for years, usually with extensive pulmonary involvement associated with cavitation and progressive change. In many respects this resembles chronic tuberculosis and sometimes is associated with it.

2. Non-fatal, Symptomatic Histoplasmosis. This group may be divided into mild, moderate and severe forms and does not differ appreciably in children and adults. Influenza-like symptoms are common with prominent respiratory manifestations. The onset is often sudden with cough and chest pain. Temperature elevation is commensurate with the general severity of illness. The diagnosis of atypical pneumonia is commonly made. Infants may have a clinical course indistinguishable from that of the fatal cases except for the final outcome, and recovery may ensue without relation to attempted therapy. In many of these patients intrathoracic calcification was demonstrated in roentgenograms following recovery. The variable degrees of illness are well illustrated in those cases included in the reports of "epidemics."[60,68f,72c,116a,119] Local lesions in individual organs occur; symptoms are then related to the site of disease.

3. Non-fatal, Asymptomatic Histoplasmosis. The largest number of case reports is found in relation to this and the preceding classification, and the proof of diagnosis is often equivocal. The increasing number of unequivocal cases, however, tends to support the validity of the diagnosis in the previous cases. Most of the information comes from epidemiologic studies in relation to histoplasmin sensitivity and pulmonary calcification. Calcification elsewhere in the body (spleen) also is significantly more frequent in geographic areas with high histoplasmin sensitivity rates than in areas of low sensitivity rates. Careful search, specifically directed toward the recognition of H. capsulatum in patients dying of other conditions adequate to explain symptoms, is proving productive.[31a] Comparable success is promised by biopsy technics. Study of serial chest roentgenograms in children who are known to have given positive reactions to histoplasmin and to have developed humoral antibodies have revealed pulmonary lesions in

asymptomatic children who subsequently develop intrathoracic calcification. It may be that the designation "asymptomatic" represents limitations of clinical diagnosis as currently practiced and that the better designation would be "inapparent infection." To any physician aware of histoplasmosis it is obvious that any of the "colds," "upset stomachs," or other benign conditions which rarely demand professional medical attention may actually represent the symptoms of initial infection with H. capsulatum.

From epidemiologic studies[72c] the incubation period of the disease is estimated as from five to fifteen days. Even with this information the case reported by Schlumberger and Service[175] of an infant dying of the disseminated disease at the age of nine and a half weeks raises the question of intrauterine infection. The infant spent two weeks in the hospital because of premature birth (eight months' gestation, birth weight 5 pounds), was home for four weeks and was admitted to the hospital in the seventh week with hepatosplenomegaly, pallor and signs of widespread infection.

A consideration of signs and symptoms in the various organ systems permits an appreciation of the protean nature of histoplasmosis. Because one classification merges so imperceptibly into the next, no attempt will be made to specify which of the signs or symptoms are related to any of our arbitrary groups. To do so would be to admit a prognostic implication which does not exist.

Respiratory System. This is probably the portal of entry in the majority of cases. In a statistical study of the frequency of occurrence of the lesions of histoplasmosis, Schulz[178a] found the lungs to be most frequently involved in children over five years of age and in adults, whereas liver and lymph nodes exceeded lungs in frequency of lesions in children under five years of age. These figures were derived from autopsied patients only and therefore probably do not reflect what clinical experience would maintain, namely, that respiratory involvement must occur in almost 100 per cent of patients. Pulmonary symptoms are reviewed in detail in the papers by Hodgson et al.[83b] and Schwartz.[179a] Cough is not uncommon. It is generally non-productive, unless there is associated cavitation. It may be associated with chest pain. Hemoptysis is rare; the expectoration of broncholiths has been described in an unproved case.[94] Some patients have demonstrated dyspnea. Night sweats have been present in occasional cases. Physical signs vary with the degree of lung involvement, as in any other infection, but more frequently than not they are totally lacking. Atelectasis, emphysema, cavitation, and so on, produce the same signs as when caused by other agents.[12,83b,96]

In adults, granulomatous lesions of the upper respiratory passages have occurred with considerable frequency. Nasal and laryngeal ulcerations of a chronic nature are repeatedly described. Hoarseness is noted as the presenting symptom in many cases.[88,199a] The lack of response of symptoms to treatment with streptomycin within one week has been considered by Hulse[88] as a means of differentiating laryngeal histoplasmosis from laryngeal tuberculosis. Pleural effusion from which organisms have been cultured has been described[6,54,78] (Case III). The diagnosis has been made by biopsy of a scalene lymph node in the fashion which has been helpful in the diagnosis of sarcoid and cancer.[97]

Gastrointestinal System. Ulcerating, granulomatous lesions of the oropharynx[41b,203] and of the perianal region[145,204a] occur in both fatal and non-fatal cases. All intervening anatomic divisions of the gastrointestinal tract have been implicated. Weakness, sore throat and dysphagia accompany lesions in the oral cavity. These lesions often spread to the upper respiratory passages. Gastric ulcer with massive hemorrhage has been described.[65] Ulcerative enteritis has been found at all levels; the clinical manifestations of such involvement, apart from the anorexia which may be systemic rather than local in origin, have been largely diarrhea and/or melena.[64,80] Icterus has been reported in some cases but is not common; hypoproteinemic edema may occur, related in part to involvement of the liver. Hypergammaglobulinemia may occur (Case II). Hepatosplenomegaly has been the hallmark of the disease in the disseminated fatal form in infants. Histoplasmosis is very likely to be the correct diagnosis in a pale, febrile child with marked hepatosplenomegaly in the endemic area. Abdominal enlargement is a common observation in infants; in adults the enlarged organs are frequently palpated but abdominal enlargement is rare. Intra-abdominal masses of lymph nodes are occasionally palpated.

Genitourinary System. Although involvement of practically all organs of this system has been observed histologically, clinical signs are largely restricted to penile, chancre-like lesions in adults[41b] and manifestations of adrenal insuffi-

ciency in all ages.[160] Classical Addison's disease has been noted in several fatal cases in adults, and histologic involvement of the adrenal is so frequent as to make histoplasmosis suspect whenever signs of Addison's disease are observed in the endemic area. No proved transplacental cases are recorded but the early age of clinical onset in some cases[175] has raised this possibility.

Cardiovascular System. Endocardial lesions have been noted at autopsy in several instances.[11a,17,19] In one case[68c] there was electrocardiographic evidence of right bundle branch block but this was not necessarily related to the local cardiac lesions present. Periarteritis has been reported;[27] subendothelial masses of parasitized macrophages[178a] might be expected to be associated with other vascular lesions.

Nervous System. The fact that lesions have been found in the brain[99] raises the interesting possibility that some of the convulsive episodes, periods of coma, and so on, may be related to local brain disease. Clear-cut localizing neurologic signs related to a distinct lesion of the central nervous system have not been recognized. Chorioretinitis may be considered a manifestation of nervous system involvement. One patient with a positive complement fixation test had clinical chorioretinitis supposedly "reactivated" by the application of a skin test which gave strong positive results.[107]

Eyes and Ears. Apart from the chorioretinitis noted, a high incidence of positive histoplasmin reactions has been observed in patients with uveitis.[44] The middle ear has been the site of histoplasmosal lesions; no diagnostic clinical features are described but the organism may be found in purulent aural discharge.[135b]

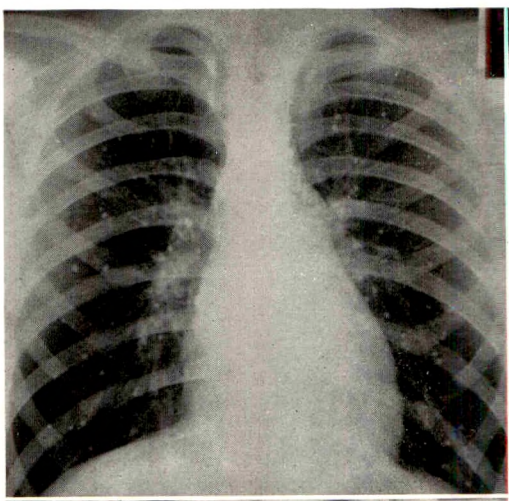
Skin. Skin nodules have been found in some cases and biopsy in such cases has established the diagnosis.[76,95,195b] In one of our cases exfoliative dermatitis was present, and following treatment with ACTH the skin became the site of intensive and extensive histoplasma proliferation. It is strange that erythema nodosum, which is common in coccidioidomycosis, has not been unequivocally present in histoplasmosis; in those cases wherein it is described either the lesions have not been typical or the possibility of another mechanism such as penicillin reaction exists.[68c,78] Rarely, granulomatous lesions develop at sites of excision of infected lymph nodes for biopsy purposes.[48] One of the authors' patients had an incision and drainage of a mass of necrotic cervical lymph nodes and, although healing was slow, it was complete.

Skeletal System. Key[101a] described clinical arthritis of the knee with osseous destruction leading to amputation. Organisms were found in the thickened synovia and in the granulation tissue of the bone. General skeletal involvement has been infrequently reported.[104,121] With the frequency of marrow involvement in the disseminated disease, however, lesions of bone must occur but are probably too small or diffuse for roentgen recognition. Infection in a sternoclavicular joint has been described[131] but apparently was associated with torula infection.

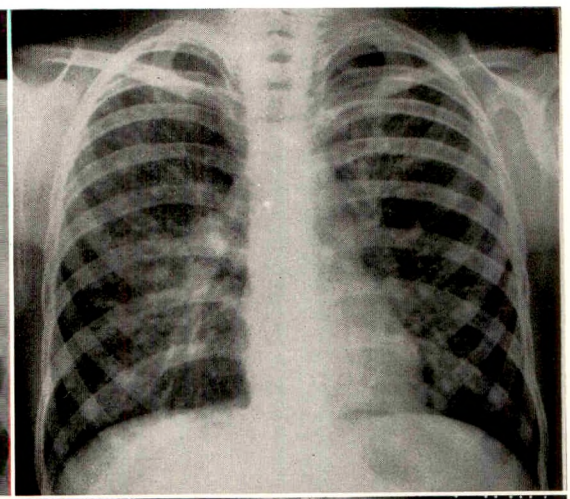
RADIOLOGIC FEATURES

Radiologic examination of the chest is one of the most important factors in calling attention to the frequency of histoplasmosis in certain areas of the country. Nevertheless, except for the recognition of disseminated intrathoracic calcifications (Figs. 14 and 15B), there are no diagnostic features which clearly differentiate radiologic signs of histoplasmosis from other pulmonary inflammatory disease. Claims for diagnostic specificity of "mulberry-like" calcifications or focal calcification with a "soft halo" do not stand critical scrutiny. Nodular foci of increased density, pneumonic infiltrations, hilar and mediastinal lymphadenopathy, disseminated infiltrates and various combinations have been observed during the acute phase of the disease.[68j, 85,187] (Figs. 15, 16 and 17.) The shadows tend to persist much as those of tuberculosis with which they are often confused. Atelectasis and emphysema of a lung or of a lobe may result from bronchial compression by involved lymph nodes. Many of the lesions go on to calcify, producing primary complexes which are indistinguishable from those of tuberculosis. (Fig. 18.) Calcification has been observed to develop in as short a time as four or five months after clinical illness.[108] Solitary lesions, "histoplasmomas," have been described and have been resected; it is not clear how certain the diagnosis of histoplasmosis was in some cases but morphologic and cultural proof has been offered in others.[126, 154] The presence or absence of calcification within the solitary lesions has no diagnostic significance. In some of the "epidemic" cases fine widespread reticulations are observed in the lungs (Fig. 17);[63a] calcification seems less common in this type of reaction than in the focal or nodular variety. Gross cavitation has

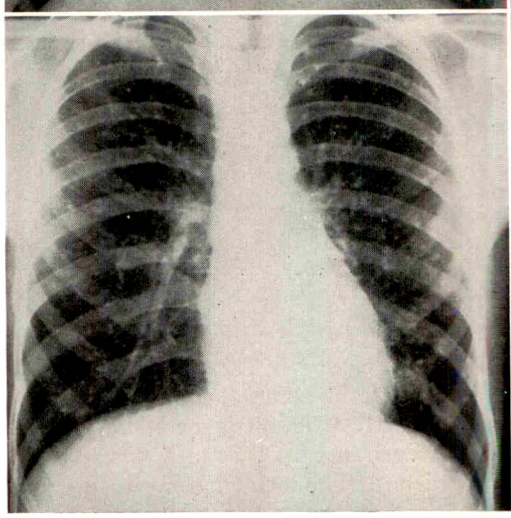
14



15A



15B



16

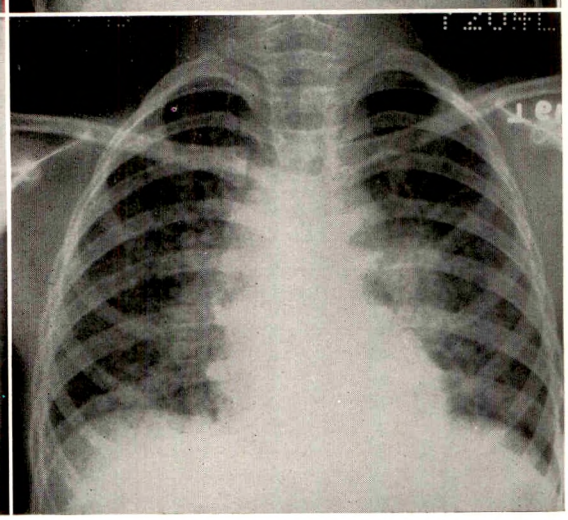


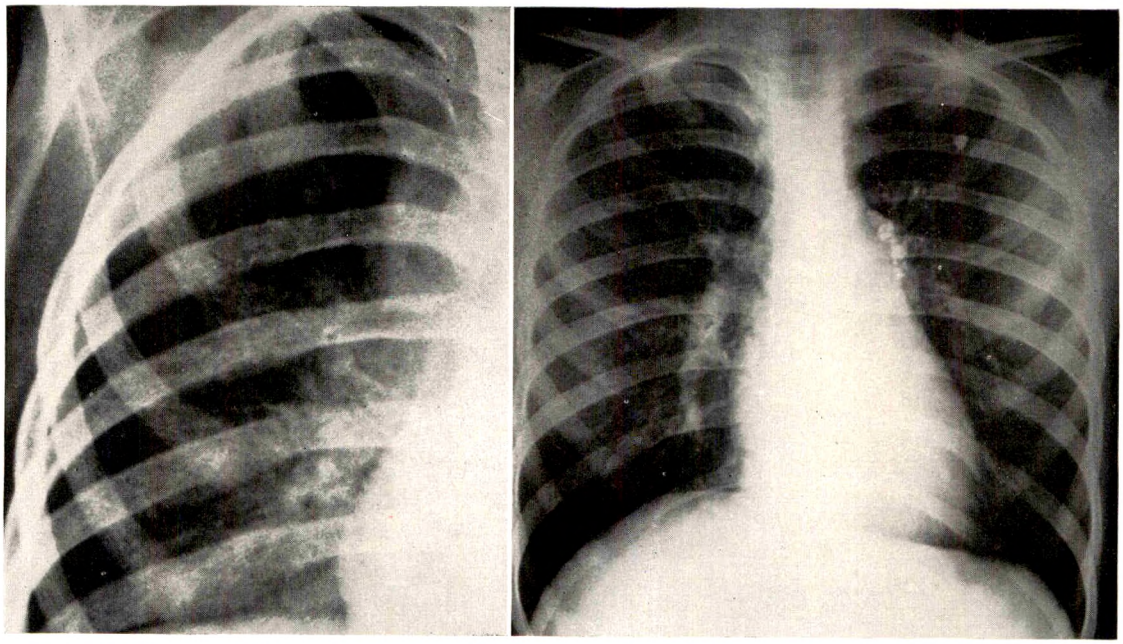
FIG. 14. Probable histoplasmosis; disseminated calcification in a boy who gave a negative reaction to tuberculin and a positive reaction to histoplasmin. No definite respiratory infection was present, yet lesions developed while the patient was under careful medical supervision for rheumatic fever. This roentgen picture is practically diagnostic of remote infection in the endemic area. (From *Am. J. Roentgenol.*, 64: 747, 1950.)

FIG. 15. A, probable histoplasmosis; acute respiratory illness; x-ray diagnosis of "miliary tuberculosis." Negative reaction to P.P.D., positive reaction to histoplasmin six months after acute episode following discharge from tuberculosis sanitarium where diagnosis of tuberculosis had been disproved. B, two and one-half years later; patient clinically well. Note beginning of disseminated calcification.

FIG. 16. Probable histoplasmosis; positive reaction to the skin test and C.F.R. This is an example of diffuse nodular pulmonary disease with hilar lymphadenomegaly. (From *Am. J. Roentgenol.*, 64: 747, 1950.)

been observed exclusively in adults, many of whom were under treatment for tuberculosis which they did not have. Others with cavitation have had both diseases, but radiologic features did not permit evaluation of the contributions provided by each separately. Pleural reactions are readily recognized roentgenographically when present.

The absence of pathologic pulmonary shadows does not exclude pulmonary involvement. The limitations of size of lesions, shape, sharpness of margins, location and total number which apply to any disease militate against roentgen recognition of disease in histoplasmosis as well. In Case 1 review of the chest film taken less than twenty-four hours before death failed to identify two well developed primary foci found on autopsy, each measuring 3 mm. in diameter and containing enough calcium to be gritty when sectioned. Although lesions are found in the thymus, enlargement of this organ due to histoplasmosis is seldom seen.[82]



17 18

FIG. 17. Proved fatal disseminated histoplasmosis in an infant; markings in the lung demonstrate a free reticular pattern; the interlobar pleura is slightly thickened.

FIG. 18. Case VII (B. J. S.); proved asymptomatic histoplasmosis. Calcifying lymph node removed during surgery for uncomplicated patency of the ductus arteriosus; many non-viable typical H. capsulatum were found. (See Fig. 37.)

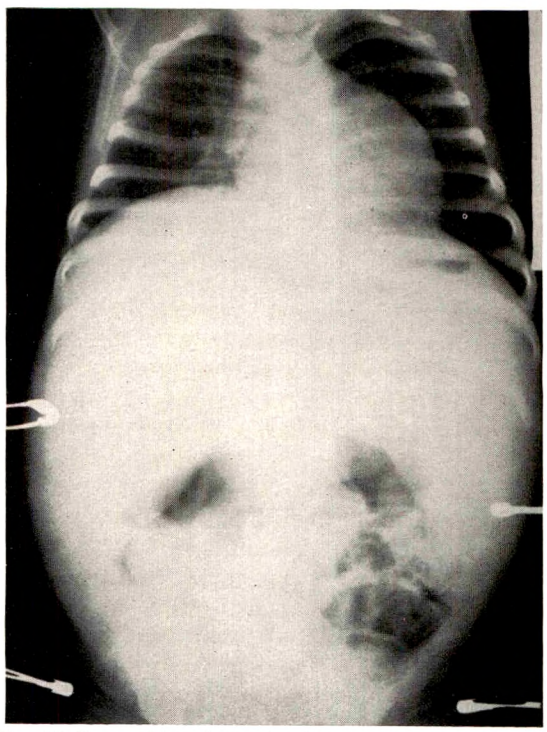


FIG. 19. Proved histoplasmosis with spontaneous recovery; note massive hepatosplenomegaly. (From *Am. J. Roentgenol.*, 64: 747, 1950.)

Films of the abdomen, or portions of the abdomen included in chest films, may demonstrate hepatosplenomegaly. (Fig. 19.) Splenic calcification was found in chest films in a few persons examined as part of a survey by High and others.[81a] The incidence of splenic calcification is not adequately portrayed by clinical roentgen examination of either the chest or the abdomen. In a study of spleens removed routinely at autopsy and radiographed, the incidence of splenic calcification in a region of high histoplasmin sensitivity rates is more than ten times that in a region where the disease is known to be practically non-existent.[180e] Other extrapulmonary calcification may occur, for example, in axillary lymph nodes, but thus far proof of infection in such cases is only circumstantial.

The bone lesion described by Key[101a] was merely spotty bone atrophy associated with soft tissue thickening about the knee and fluid in the joint. No productive changes were present. Granulation tissue with cells containing parasitized organisms was found to be invading the bone in the area where the demineralization was noted.

Radiologic examination of other body sys-

tems has not been reported as helpful. Undoubtedly, ulcerations of the gastrointestinal mucosa might be identified in some cases; perhaps contrast studies of the large bowel in those cases with colitis or of the small bowel in those with enteritis may prove of value.

DIAGNOSIS

The diagnosis of histoplasmosis must be based on the demonstration of organisms, with preference by culture. The indirect methods like complement fixation will only under very special circumstances replace a culture as acceptable proof. Frequently, especially in endemic areas, the decision as to whether or not a case can be classified as histoplasmosis may be very difficult in the absence of a positive culture.

The morphologic diagnosis of H. capsulatum in tissue can be accepted only seldom and only in the hands of experienced observers. The similarities with Histoplasma farciminosum and organisms of the leishmania group have been mentioned repeatedly. Furthermore, cell debris especially in lymph follicles is indistinguishable from isolated organisms, and in every appendix or tonsil structures can be found which resemble but cannot be proven to be organisms. Misinterpretation of such débris as histoplasma has been repeatedly reported.[156a,b,208]

Unfortunately, and for obvious reasons, it is hard to estimate how often organisms are missed. We have often searched unsuccessfully for histoplasma in proved cases for hours. We found little help from periodic acid stain in locating a small number of organisms; however, the Gridley procedure[73b] offers so much contrast that it contributes greatly to the search for fungi in tissues. On the other hand, observations like those of Raftery which report the organism in 5 per cent of random samples of appendices are open to question. His work has been checked by reliable observers and by cultural methods, and the presence of histoplasma in normal appendices has not been established.[32,169]

Histoplasmosis, as we see it, is a disease which is acquired by way of the respiratory tract almost without exception. As in most granulomatous diseases, an overwhelming majority of persons affected with the primary infection are asymptomatic and the episode is self-limited. The arrest of the primary infection is frequently permanent, and its recognition is often limited to radiographic demonstration of calcific chest lesions. Rarely, the disease can become progressive or reinfection may occur. It is not possible at our present level of information to say whether chronic, active (often cavitary) histoplasmosis is derived directly from the primary infection or whether it represents reinfection. If the latter, by analogy with well established facts in tuberculosis, endogenous or exogenous mechanisms could be involved. It seems fairly certain from the Cincinnati epidemic[170] that heavy exposure may produce exogenous reinfection; how often endogenous reinfection occurs is not known at present. The isolated tuberculoma-like lesions, which are found in the lungs with increasing frequency and which are being resected as "histoplasmomas," may represent foci of lymphatic, hematogenous or even bronchogenic reinfection. We have no knowledge of the effect on the disease of sensitization, nor of what mechanisms are responsible for encapsulation on the one hand or dissemination on the other. It would indeed be of paramount importance to obtain information on these points.

We recommend the following step-by-step procedure in a case of suspected histoplasmosis: (1) *Skin test.* If the results are positive, the possibility exists that the disease is histoplasmosis. Special care must be taken with respect to proper dilution, potency and sterility of the antigen. If the results are negative, the possibility of the presence of histoplasmosis is remote unless the patient is severely ill or in the incubation period. (2) *Complement fixation test.* The difficulty of obtaining a reliable test cannot be overestimated. A positive reaction to the test with high titer is generally indicative of progressive disease but cross reaction should be ruled out. Low titer or negative reactions do not rule out active disease. (3) *Biopsy* of enlarged lymph nodes, mucocutaneous lesions, liver, and so on. (4) *Culture from blood and bone marrow.* (5) *Culture of material from organs producing symptoms*, sputum or gastric washings, skin exudate, feces, urine (all contaminated material mixed with antibiotics should be injected into the peritoneal cavity of mice).

It cannot be overemphasized that the demonstration of the organism in blood smears, bone marrow, tissue, sputum, and other sources, requires skill which cannot be expected from every laboratory, especially outside the endemic area. This refers both to microscopic and cultural demonstration. The statement is borne out by the amazingly small number of cases which

would fulfill even modest minimal standards of diagnosis.

TREATMENT

It is quite obvious from the extreme variation in the clinical course of the disease that evaluation of any therapeutic agent must be extremely difficult. To date no specific therapy has been developed. Supportive treatment in the form of dietary measures, food supplements, transfusions, effective treatment for underlying or superimposed disease, is governed by considerations unrelated to the presence of histoplasmosis, and the indications are well known and clearly defined.

The rapidly progressive, disseminated cases of histoplasmosis seen especially in childhood (Case I) compare with the disseminated cases observed in tuberculosis as a consequence of primary infection at a time when body defenses have not been mobilized or have proved to be inadequate. Paradoxically, one would expect these cases to be most benefited by chemotherapy if and when such therapy becomes available. In the disseminated cases the problem would seem to be one of fungicidal therapy. The chronic cases may not need eradication of the organisms so much as a general change of the internal environment. The internal environment probably is altered initially by the fungus, becomes less important in the granulomatous forms of the disease, but assumes a significant role as in Cases III and IV when sensitization, perhaps, permits extensive proliferation of the organism.

Specific drugs which have been used in individual cases of histoplasmosis or evaluated *in vitro* include arsenicals, sulfonamides, iodides, organic fungicides, antimony compounds, antibiotics, and so on. Radiation therapy and surgical resection have been described.

Arsenicals. Various arsenical preparations have been tried since the earliest case reports. Reports of success in the treatment of epizootic farciminosis with arsenicals naturally led to its use in histoplasmosis, the causative organism of which may be closely related to H. farciminosum.[184] Seabury and Artis[182b] found neoarsphenamine effective against H. capsulatum *in vitro* in a concentration of 100 mg./100 cc. It is doubtful whether levels of this magnitude could be reached and maintained safely in tissue fluids.

Sulfonamides. The availability of these chemotherapeutic agents coincided with the recognition of histoplasmosis as a not so rare disease. Various members of this family have been used in individual cases or very small series with variable results.[41b] Sulfanilamide and sulfadiazine were thought by some to influence the disease but experimental studies would seem to indicate that these drugs in accepted dosage would not be effective.[113,182b]

Organic Fungicides. At least two preparations used to inhibit fungus growth in commercial enterprises (baking) have been given fairly intensive study. Christie[31c] reported treating twelve infants with the disseminated form of the disease with ethyl vanillate; five children recovered but the relationship between administration of the drug and the recovery is far from proved. Toxicity of this agent is great and, in general, further use of the drug in its present form is not recommended. Sodium propionate has been studied under experimental conditions;[113d] it does inhibit the growth of the organism but clinical success has not been reported.

Antimony Compounds. Certain clinical and pathologic similarities of histoplasmosis and kala-azar undoubtedly led to consideration of antimony compounds in the former disease by virtue of its long association with the latter. Fuadin® and neostam® have proved ineffective in *in vitro* studies.[39,113a,182b] H. capsulatum was susceptible to stilbamidine *in vitro* in concentrations between 10 to 100 mg./100 cc.[182b] but subsequent clinical trial was unsuccessful in influencing the course of the disease.[182a] Because of apparent success in the treatment of blastomycosis,[189] 2-hydroxystilbamidine has been offered as a possible antihistoplasmal agent.[182a] In one case (Case II) the drug was not clearly beneficial and may have been toxic. Meleney[127] mentions clinical remission during neostam treatment; other reports of the clinical use of antimony are not encouraging.

Antibiotics. None of the regularly available antibiotics has been found to affect H. capsulatum. In fact, through inhibition of various other microorganisms, these agents may actually enhance the invasive and pathogenetic properties of H. capsulatum.[182b,c] Experimental studies have identified at least one laboratory antibiotic which may have some effect. Fungicidin, obtained from a soil actinomycete by Hazen and Brown,[77a] is reported to be active against H. capsulatum *in vitro*, as well as against Candida albicans and others. This antibiotic, also known as nystatin,® has given promis-

ing results in experimental histoplasmosis in animals.[22e,52a,77c] Hulse[88] regarded the lack of response of laryngeal lesions to streptomycin as a test differentiating histoplasmosis from tuberculosis.

Miscellaneous. Atabrine® was found experimentally to inhibit the growth of H. capsulatum *in vitro*.[22d] Experimentally infected mice showed increasing survival rates with increasing dosage schedules. Organisms were present in the spleen of both treated and untreated mice, indicating that the effect of atabrine was suppressive rather than lethal. Treatment begun one week after infection was ineffective in reducing mortality rates. Hansen and Bean[75] found a abrine ineffective at concentrations which generally produced effects. Iodides were ineffective *in vitro*[113a] and had no clearly defined clinical effect.[118b,135b]

Radiation therapy has been used for some of the localized lesions[80,147b] and for splenomegaly.[20] Results have been equivocal; local lesions may respond. However, Brandt[16a] believed that radiation enhanced experimental infectivity in mice and warned against x-ray therapy in human disease. A comparable warning, resulting from a somewhat similar mechanism, may be read in Cases III and IV with regard to the use of cortisone. The apparent run-away spread of the disease in our two patients so treated may constitute an additional contraindication to indiscriminate use of such medication in an endemic area of histoplasmosis. Experimental histoplasmosis in mice did not, however, become disseminated after cortisone injections.[9]

Certain antihistaminics were found to exert an inhibitory effect on H. capsulatum, among other fungi, in experimental studies but effective concentrations are beyond the range of clinical usage.[24]

Surgical incision of local lesions might be expected to yield a cure in the absence of disease elsewhere in the body. Recent interest has been raised with respect to excision of pulmonary lesions.[12,83a,126,154,213] In tuberculosis, in which resection has become popular, chemotherapeutic and antibiotic auxiliaries are available; in the absence of such additional support in histoplasmosis considerable reserve must be maintained with regard to surgical therapy. Splenectomy has been reported in several cases.[5,20,144] The indication has generally been marked splenomegaly and "hypersplenism." Recovery and death have been reported but it is interesting to know that recovery can occur when hypersplenism is relieved. A report of improvement after administration of acti-dione® is available.[207] Negroni[139a] speaks of a vaccine but his report is open to considerable question.

Vaccination has been shown to be effective for short intervals in mice[171h,173] but a prophylactic program in humans does not seem feasible. The disease is one in which infected individuals seldom become obviously symptomatic so that evaluation of protection would be extremely difficult if not impossible. Moreover, repeated injections are necessary to maintain immunity, and no clear criteria are available for selection of any particular group as most desirable for vaccination. Most "natives" in the endemic areas apparently acquire the disease even at school age; their skin sensitivity may be related to natural immunity. Newcomers to the area might be considered at greater risk from histoplasmosis but it is clear that many adults who enter an endemic area remain permanently nonreactive to histoplasmin. Prevention of the disease is difficult to visualize if the concept of soil contamination and inhalation of dust proves to be correct. Menges' report[129g] of finding H. capsulatum in the soil while searching for blastomyces in a place where a dog was found with blastomycosis serves to illustrate how widespread is the contamination of soil in the endemic area.

Hansen and Beane[75] report no effective *in vitro* action by a variety of agents including certain chlorinated hydrocarbons, quinine, emetine, aminopterin, PASA and urethane. Currently, beta-di-ethylaminoethyl fencholate is under investigation.[117]

We are frequently confronted with the problem as to whether or not an individual with primary disease diagnosed by change in skin sensitivity and/or radiographic changes should be kept in bed. Likewise, the question of prolonged bed rest is encountered in cases of cavitary disease. Too little is known at present about the natural course of the disease to make any fast rules about the management of patients but it does seem reasonable to say that primary infection does not require bed rest once the acute febrile episode and respiratory symptoms have subsided. No cases of generalized disease have been traced to overexertion or overexposure. Chronic cavitary lesions probably require bed rest only as part of the general supportive therapy. Whether or not in the future surgical

procedures like pneumothorax, thoracoplasty, and so on, will be shown to be helpful in accelerating closure of cavities we do not dare prognosticate. Since no evidence has been presented that resection of isolated pulmonary lesions conclusively improved the prognosis in tuberculosis, it is obvious that resection of isolated pulmonary lesions in the less well studied histoplasmosis is far from an established therapeutic procedure. Naturally, surgery will be indicated and permitted in all cases in which the slightest doubt exists concerning diagnosis, and in which the possibility of malignant tumor must be considered. It may be an important consideration that no drugs are available at present to safeguard patients from extension of infection after surgery in histoplasmosis the way streptomycin and allied drugs provide an "umbrella of protection" in tuberculosis.

CASE REPORTS

CASE I. C. S., born December 30, 1950, was admitted to the hospital on December 16, 1952, because of a low-grade fever of insidious onset and loss of appetite of three weeks' duration, and a high fever for four days prior to admission. The patient did not respond to treatment with antibiotics.

The birth, development, feeding and past medical histories were non-contributory. The family history was interesting insofar as a female sibling was admitted to the hospital December 18, 1952, and subsequently proved to have disseminated histoplasmosis (Case II). Epidemiologic aspects will be the subject of another communication.[1]

Physical examination at the time of admission revealed a somewhat pale girl who appeared both acutely and chronically ill. The weight was 18 pounds, 4 ounces; temperature 103.4°F., pulse rate 160; respiration rate 100. Small cervical lymph nodes were present, the liver was easily palpable four fingerbreadths below the right costal margin and the spleen was easily palpable two and one-half fingerbreadths below the left costal margin. There were rhonchi and scattered rales through the lung fields and a marked tachycardia was present. Both tympanic membranes as well as the pharynx were moderately reddened. The remainder of the examination revealed essentially no abnormalities.

The patient's course was a stormy one, characterized principally by high spiking fever ranging between 101°F. and 107°F., by rapid respiratory rate with intermittent cyanosis, frequent passage of large bulky black stools, recurrent nose bleeds and bleeding from the site of venipunctures and during the terminal stages by vomiting of dark blood. There was no apparent response to various forms of therapy which included blood transfusion, intravenous fluids, aureomycin® and penicillin, and one intravenous injection of 2-hydroxystilbamidine. The patient died on December 19th, three days after admission.

During her hospital stay the following laboratory data were noteworthy:

1. The blood count on December 17, 1952, revealed a hemoglobin of 5.3 gm., red count of 2.84 million, reticulocyte count 3.0 per cent, platelets 5,600 per cu. mm., white count 4,100 per cu. mm., with 78 per cent neutrophils, 2 per cent eosinophils and 20 per cent lymphocytes. There was marked hypo- and polychromasia and poikilocytosis. Occasional H. capsulatum were found in the peripheral blood. There were 40 normoblasts per 100 white cells. The sedimentation rate was zero; mean corpuscular volume 74, mean corpuscular hemoglobin 18, mean corpuscular hemoglobin concentration 25.

2. Urine showed a 1+ to 2+ albumin with occasional white cells on microscopic examination. Specific gravity ranged between 1.009 and 1.015.

3. H. capsulatum was cultured from both the blood and the bone marrow on December 17, 1952. The marrow was heavily parasitized with H. capsulatum. (Fig. 20.)

4. Roentgenogram of the chest revealed exaggerated markings in both lung fields with a slight pleural reaction on the right. Review of films in the light of necropsy findings failed to permit roentgen identification of the primary foci.

Autopsy. Externally there were no superficial lymph node enlargements or edema. Pertinent findings were limited to the internal examination.

The pleural surfaces were dotted with petechiae. Delicate fibrous adhesions were present between the right upper and right middle lobes. The right and left lungs weighed 180 and 140 gm., respectively. In the subpleural region of the right middle lobe were two separate nodules, each measuring 3 mm. in diameter. They were firm, discrete and associated with fibrinous pleural thickening. When sectioned

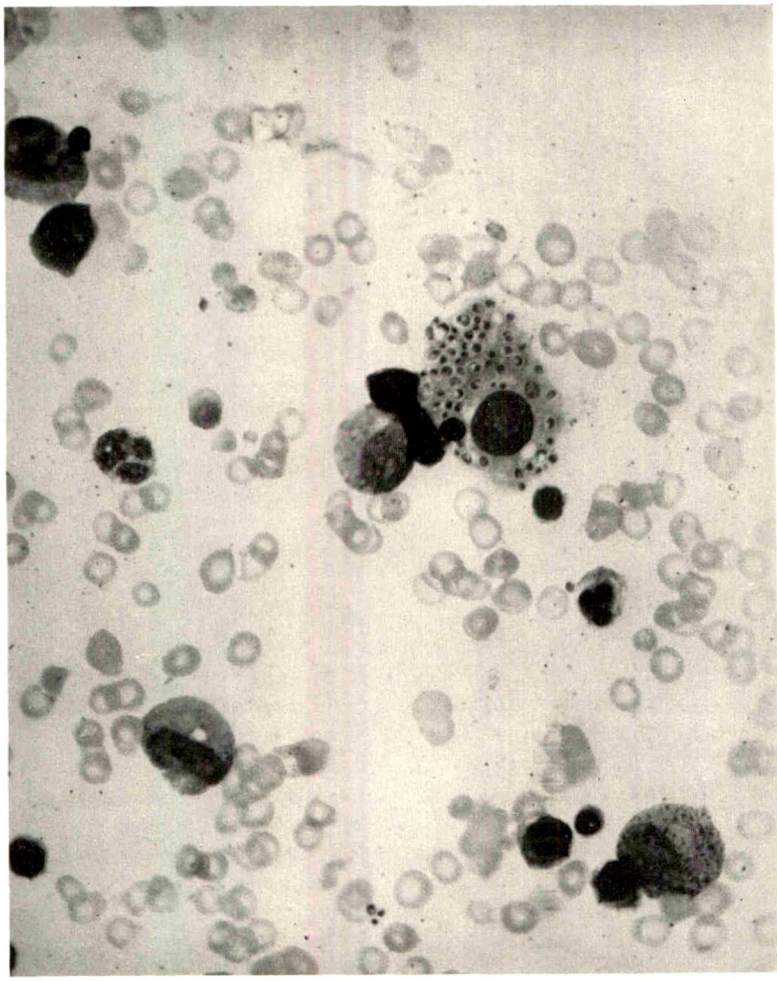


FIG. 20. Case 1. Bone marrow smear; numerous intracellular organisms and isolated group of three extracellular yeasts in lower part of field; original magnification × 650.

they were yellow, soft and contained enough calcific material to be gritty. There was a matted mass of lymph nodes at the right hilus measuring 2.5 by 1.5 cm. Individually they were nodular, firm, white and with stellate areas of yellow necrosis, resembling caseation. Calcification was not apparent. The other tracheobronchial lymph nodes were slightly enlarged but free from focal change. The lung substance was diffusely non-crepitant and consolidated. Sections revealed reddish-tan, firm, granular tissue which was somewhat friable. The process was most prominent in the posterior and apical segments of the right upper lobe.

The liver weighed 950 gm. and extended 6 cm. below the costal margin. The cut surface was yellow-tan, pale and mottled. An occasional grey nodule, 1 mm. in diameter, was noted.

The spleen weighed 150 gm. Scattered grey tubercles, to 1 mm. in extent, were visible on the capsular surface. Sections revealed occasional small irregular areas suggestive of confluent granulomas. Two accessory spleens, 1 cm. in diameter, showed similar change.

Throughout the small intestine and colon, most prominent in Peyer's patches, were follicular areas of erosion and hemorrhage. They were firm, considerably elevated and up to 2 mm. in diameter. The appendix revealed no abnormality. In the transverse colon were four polypoid lesions 1 cm. in greatest extent. They were firm, ovoid and button-like. Central erosions were coated with hemorrhage and a membranous exudate. The colonic wall in this area was thickened, indurated and adherent to a matted mass of mesenteric lymph nodes measur-

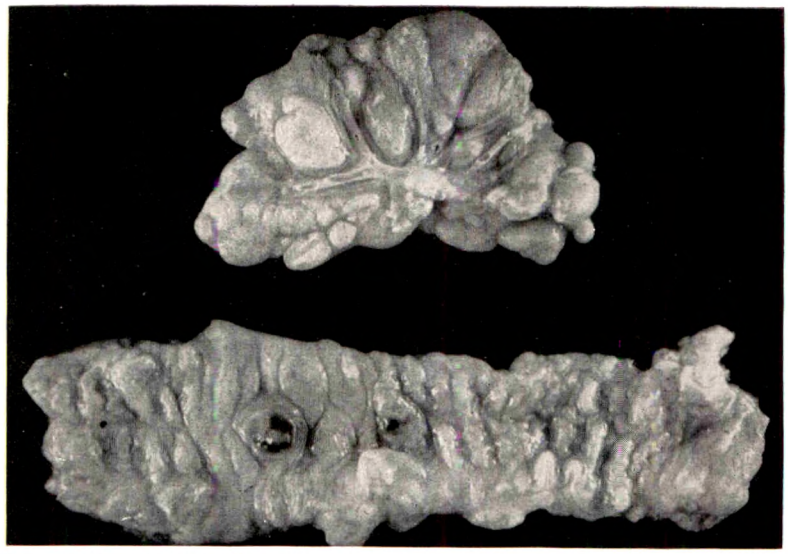


FIG. 21. Case I. Ulcerative colitis and mesenteric lymphadenopathy in presence of typical pulmonary primary complex.

ing 15 by 12 by 3 cm. These and confluent groups of periaortic and peripancreatic nodes resembled the tracheobronchial nodes when sectioned. (Fig. 21.)

The bone marrow and the adrenals revealed no abnormalities upon gross examination.

Microscopic Examination. The two subpleural nodules were of similar appearance. Central areas of necrosis and early calcification were rimmed by dense collagen and very little inflammatory reaction except for a few lymphocytes and giant cells. (Fig. 22.) Throughout the lung there was thickening of alveolar septa and interstitial fibrosis. Infiltration of histiocytes and lymphocytes, and occasional thrombosed capillaries were seen in the framework. Extruded into alveolar spaces and often packing them were large numbers of histiocytes and multinucleated giant cells. Within the nodules and also within the alveolar histiocytes were innumerable organisms (H. capsulatum). In tracheobronchial, mesenteric, pericolic and retroperitoneal lymph nodes there were nodular areas of caseation necrosis intermixed with collagenous scarring, but little tubercle formation or giant cell reaction was noted. Thousands of fungi were seen.

In the spleen there was a diffuse infiltration of the cords and sinusoids by great numbers of histiocytes, all of which were packed with H. capsulatum. Necrosis or frank tubercle formation was not seen.

Throughout the lobules of the liver, irregular collections of histiocytes were present, sometimes in small nodules. Minute foci of necrosis with early fibrosis and a few multinucleated giant cells were occasionally encountered. Again, a multitude of fungi was seen.

The involvement by H. capsulatum of the intestine and colon had a distinct predilection for the lymphoid tissue. The scattered mucosal follicles and Peyer's patches were the site of nodular aggregations of histiocytes which were packed with fungi. (Fig. 23.) On the mucosal aspect there was necrosis, ulceration and microabscess formation. The larger polypoid lesions in the transverse colon were an exaggeration of this change. Also encountered in the larger lesions were foci of acute necrosis and bacterial invasion extending into the pericolic fat.

One adrenal revealed a single minute nodular infiltrate of histiocytes, containing fungi.

The bone marrow revealed a diminished content of hemopoietic elements although there was a recognizable erythroid hyperplasia. The striking feature was a diffuse and intense infiltration by histiocytes which were packed with H. capsulatum. Necrosis or tubercle formation was not seen.

CASE II. M. S., a white female, four years and one month of age, was admitted to Children's Hospital on December 17, 1952, when it was learned that she was not well and had swollen glands in her neck. (Fig. 24.) Her



22



23

FIG. 22. Case 1. Primary focus of the lung; well circumscribed necrotic focus with fibrous wall and slight pleural involvement; original magnification × 17.

FIG. 23. Case 1. Mostly diffuse mononuclear cell infiltrate of mucosa and deeper layers of small bowel; note absence of true ulceration, indicating that the infiltrate is not due to infection from the intestinal lumen. Original magnification × 150.

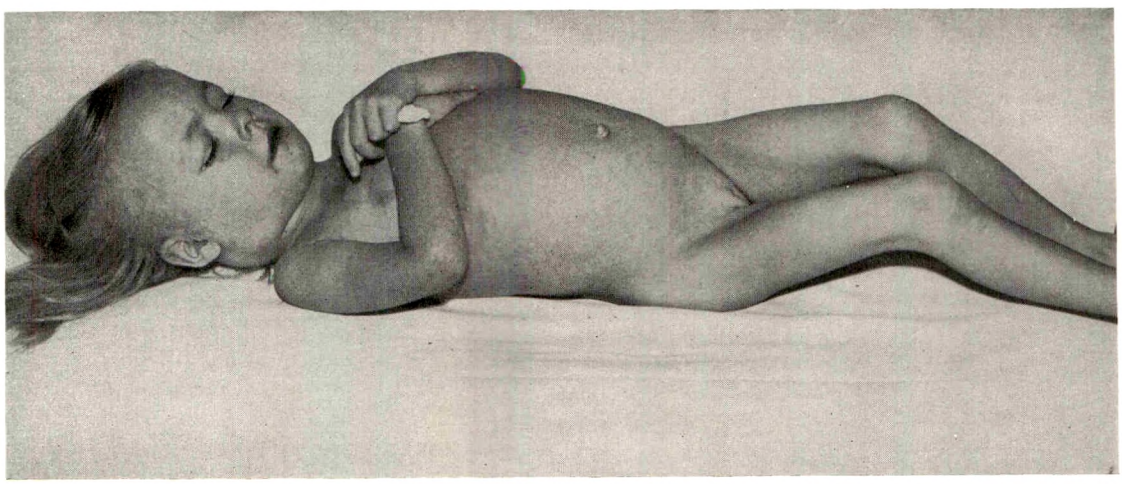


FIG. 24. Large cervical lymphadenopathy; stupor; abdominal swelling due to ascites, lymphadenopathy, hepatosplenomegaly and meteorism. Note atrophic lower extremities.

sister, C. S., admitted the day before, had been found to have acute disseminated histoplasmosis, proved by examination of the bone marrow.

Both children became ill approximately three weeks before admission to the hospital. M. S. developed progressive painless enlargement of cervical lymph nodes and slight cough; there was no change of appetite, which had been generally poor, no recognized fever, weight loss, abdominal enlargement or other evidence of systemic disease.

Physical examination on admission revealed a

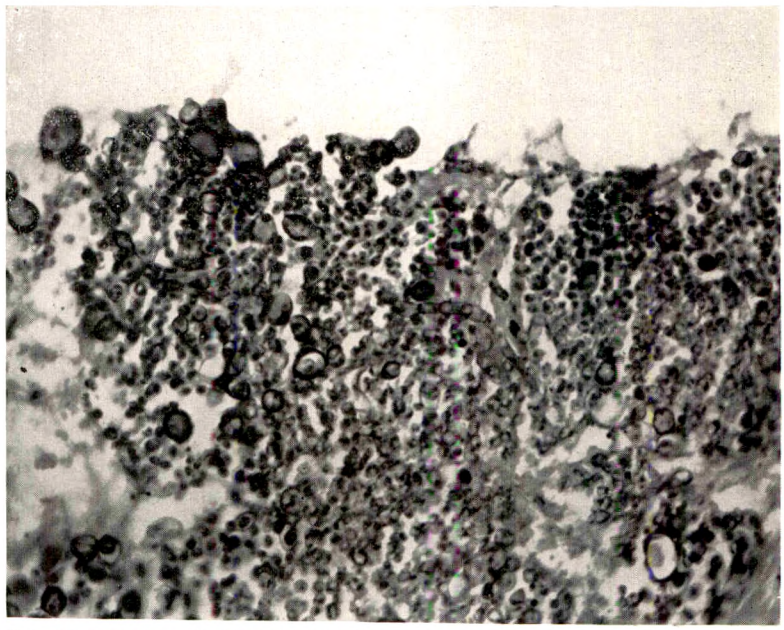


FIG. 25. Lymph node (Case II); small and large cells of Histoplasma capsulatum close to surface of tissue explant after ten days; original magnification × 650; periodic acid Schiff stain.

thin, poorly developed and poorly nourished girl who appeared chronically ill. Massive enlargement of lymph nodes on both sides of the neck resembled the classical picture of Hodgkin's disease in adults. The masses of nodes were matted together, were firm and non-tender and were not adherent to the overlying skin, which was not reddened. Nodes of 1 to 3 cm. were present in the axillary and inguinal regions and were firm and non-tender. Except for a soft systolic murmur heard by one examiner, the examination of the heart and lungs was not remarkable. The abdomen was distended, the liver and spleen were both easily palpable. Irregular masses, thought to be enlarged intra-abdominal lymph nodes, could be palpated in the abdomen, particularly in the right lower quadrant. The patient was conscious but irritable.

Laboratory examinations were undertaken specifically with respect to isolation of H. capsulatum. Blood cultures taken on the following dates were all negative: December 18, 23, 24, 28, 1952 and January 5, March 24, 1953. Bone marrow aspiration of the spinous process and sternum performed on December 18, 1952, and January 9 and March 25, 1953, revealed all smears to be negative; cultures were positive after each aspiration except on January 9th. Examination of the stools on April 7 and 8 and May 12, 13, 14, 1953 revealed them all to be negative for H. capsulatum. Cervical nodes (Fig. 25) showed the following: A biopsy performed on December 29, 1952 revealed smear, culture and section to be positive; aspiration performed on February 4, 1953 revealed the culture to be positive; incision and drainage were performed on February 8, 1953; drainage swab on March 16, 1953 revealed the culture to be positive, drainage swabs on April 8 and April 27, 1953 gave negative results from the cultures.

Other laboratory procedures and their results were: blood count on admission: 8.2 gm. hemoglobin; 3.94 million red blood cells; 2.24 per cent reticulocytes; 230,000 platelets; 9,100 leukocytes; 71 per cent polymorphonuclears; 1 eosinophil; 23 lymphocytes; and 5 mononuclears. Until April, 1953, there were several cycles of decreasing hemoglobin levels responding to transfusion; subsequent decreases of hemoglobin levels were irregular and relatively precipitate following episodes of massive gastrointestinal hemorrhage.

The white blood count ranged between 13,000 and 11,000 for the first three weeks. A marked leukopenia occurred on January 8, 1953, but by January 27th the white blood count had attained a level of 13,800 per cu. mm. Thereafter the white blood count ranged be-

tween 7,500 and 16,000. On March 17, 1953, there was a sharp rise to 30,700, after which time, except for occasional rises in the thirty thousand range, the count tended to run between 14,000 and 20,000. The differential counts during this period were not remarkable; the fluctuations were reflected chiefly in the percentage of polymorphonuclear cells which reached 91 per cent when the total count was in the thirty thousands. Smears of the blood at all times showed aniso- and poikilocytosis, hypochromia in the red blood cells and toxic granules and frequent immature forms in the white blood cells. No H. capsulatum were observed in any of the peripheral blood smears.

Urine examinations indicated normal concentrating and diluting power. Albumin, bacteria, white blood cells and occasional casts were generally present. Cultures were positive for Escherichia coli, paracolon organisms and nonhemolytic streptococci on many occasions. Proteus morgagni was isolated from the stools, which contained both gross and microscopic blood. Examination of the cerebrospinal fluid on four occasions revealed no abnormalities. Chemical studies of the blood were not remarkable except with respect to the protein fractions. The results are indicated below:

Date	Total Protein (gm./100 cc.)	Albumin (gm./100 cc.)	Globulin (gm./100 cc.)
12/22/52	7.9	3.6	4.3
1/5/53	6.6	...	...
1/20/53	7.4	3.0	4.4
2/10/53	7.0	2.5	4.5
3/16/53	6.8	2.9	3.9

The cephalin flocculation test was 3+ on December 22, 1952, but gave negative results from several determinations thereafter. On December 28th electrophoresis of the plasma revealed the following composition of the protein fractions: albumin 37 per cent, alpha-1 7 per cent, alpha-2 14 per cent, beta 14 per cent, and gamma more than 28 per cent.

Skin sensitivity to purified protein derivative and to histoplasmin (Lilly) in dilutions of 1:1000 and 1:100 was consistently negative. Complement fixation test for histoplasmosis gave positive results in a dilution of 1:512 on December 18, 1952, while the precipitin test gave positive results in a 1:2 dilution.

Clinical Course. Although proof of the diagnosis in M. S. was not obtained until later, isolation of H. capsulatum from the marrow of her sister made the clinical diagnosis of histoplasmosis almost certain. Therefore, this patient was started on a course of treatment with 2-hydroxystilbamidine* on December 19, 1952, the day after admission. Transfusions were administered practically simultaneously because of the severe anemia. For about three and one-half weeks the child had daily afternoon and evening temperature elevations generally spiking to 104°F. She was withdrawn and relatively quiet but otherwise the sensorium was normal. The cervical glands continued to enlarge. On December 29th four lymph nodes were removed from the left side of the neck; direct smears were positive for H. capsulatum and subsequent cultures and histologic sections gave positive results. About the end of the third week in the hospital she was given another transfusion of fresh, citrated blood from her uncle whose skin test for histoplasmin had given strong positive results. At the same time the dosage of 2-hydroxystilbamidine, which was administered intravenously originally and then intramuscularly, was increased to 120 mg. again by the intravenous route. The following day the child became suddenly worse, the temperature rose to 107°F. and mottled cyanosis and dyspnea appeared.

In the next three to four days, after the administration of procedures designed to reduce temperature and the use of an oxygen tent, a rather surprising improvement took place. The temperature continued to be intermittent but the maximal temperature reached was generally between 100° and 101°F. At the same time the clinical appearance of the child improved. This clinical improvement was maintained, except for progressive enlargement of the cervical lymph nodes, until the end of the sixth hospital week when a gradual increase in the amplitude of the temperature swings was noted. Early in the eighth week the lymph nodes in the neck became fluctuant and this was confirmed by aspiration. H. capsulatum were still present. 2-Hydroxystilbamidine, which was discontinued after the acute distress of the fourth week, was resumed in smaller dosage by intramuscular

* Material and guidance supplied by Dr. I. Snapper, N. Y.

injection. The cervical incisions were secondarily infected with H. staphylococcus aureus. Antibiotic therapy was instituted.

The patient continued to run the low grade septic temperature noted before incision and drainage of the cervical nodes. The mass in the right lower quadrant gradually enlarged; the child slowly became more irritable. She was not losing weight but no weight gain could be noted, and in fact she seemed to be slowly losing ground. She became progressively lethargic; at the end of the thirteenth week generalized twitching suddenly developed and the patient became unconscious. Convulsions occurred, some generalized, some unilateral, first on one side and then the other. There were short periods of lucidity but the sensorium clouded from that point on and tube or intravenous feedings were necessary. No localizing signs could be demonstrated and several examinations of the cerebrospinal fluid failed to demonstrate any pathologic change in its constitution.

Shortly thereafter, blood began to appear in the stools in large amounts. Earlier small amounts had been noted on occasion but these constituted massive hemorrhages and required transfusion. The draining incisions in the neck gradually healed.

The subsequent course was one of slow, relentless deterioration and death occurred August 25, 1953, eight months after admission to the hospital.

Autopsy. On external inspection the body was seen to be markedly cachectic and emaciated. Suppurative lesions were noted on the scalp and neck, with similar findings on the tongue. The pulmonary parenchyma showed little abnormality except for an occasional focus which will be described later. The regional lymph nodes were enlarged and showed varying degrees of fluctuation suggestive of caseation. Heart and spleen were without abnormalities. The abdominal cavity contained 200 cc. of serous turbid fluid and marked enlargement of the lymph nodes was noted. They varied in size from 5 to 25 mm. in diameter, were soft, and on sectioning showed marked formation of caseous foci. The liver was somewhat enlarged but without recognizable disease on cut surface.

There was a diffuse inflammatory process involving the entire small and large intestine. The small bowel, particularly in the ileum, had a diffuse pseudomembranous inflammation of yellow appearance. Focal areas of ulceration were seen, varying in size from 2 to 15 mm. in diameter. These ulcerations showed a tendency to encircle the intestine in a transverse manner. The ulcerations were undermined, had rolled edges and a yellowish necrotic base. The terminal ileum at the ileocecal valve and cecum showed hyperplastic granulomatous lesions of submucosa which often protruded into the lumen. These lesions showed no ulceration. The appendix was edematous, diffusely inflamed and showed marked suppuration.

The rest of the organs, including brain, showed little abnormality upon gross examination.

Microscopic Examination. In the lung a fibrocaseous focus of 3 to 4 mm. diameter was found, with beginning calcium deposits in the center; the focus was surrounded by a thin fibrous capsule. Within the capsule there was a small rim of giant cells and occasional epithelioid cells. Occasional histiocytes contained H. capsulatum which, however, were absent in the central necrotic parts. Some of the giant cells also contained organisms. In addition to this focus, there were small groups of histiocytes in the interstitial and in the interalveolar space of the different lobes of the lung containing small numbers of organisms. These groups did not form tuberculoid structures and no epithelioid cells were found.

In the small bowel necrotic surfaces were seen with occasional ulceration. Very little exudative inflammation was noted, but numerous histiocytes with large numbers of H. capsulatum were seen in all layers. In other parts, there was extensive pseudomembranous inflammation with many polymorphonuclear leukocytes on the surface; below this layer followed one of histiocytes and, finally, lymphocytes predominated. Some of the ulcers have undermined edges. In still other parts, large tubercles of histiocytes were found containing innumerable organisms. These nodules produced bulging of the overlying mucosa which in parts was ulcerated. Mesenteric lymph nodes showed extensive epithelioid tubercles with necrosis (incomplete) and numerous organisms. In the colon the submucosal histiocytes formed tubercles containing numerous organisms, often without ulceration of the surface mucosa.

The kidneys, ovary, brain, pituitary and heart did not contain lesions related to histoplasmosis. There were some foci of semi-necrotic histiocytes containing H. capsulatum in the pancreas. No

nodule formation was noted. The spleen was congested but no organisms or reaction to organisms was noted. The bone marrow was markedly hyperplastic without evidence of histoplasmosis. In the liver there were small granulomas composed of histiocytes with acidophilic cytoplasm but no recognizable organisms were found in the substance of the liver. There were focal degenerative changes in the brain without recognizable relation to histoplasmosis.

Comments on Cases I and II. These children represent the fulminating (Case I) and chronic progressive (Case II) forms of the disease as seen in childhood. Both children showed primary pulmonary complexes suggesting the respiratory portal of entry. Widespread dissemination of infection was the rule and massive gastrointestinal hemorrhages were serious complications in both children.

CASE III.* A. B., a seventeen year old, well developed, well nourished white female was admitted to the Jewish Hospital for the second time on October 31, 1952, with a chief complaint of generalized skin eruption.

The illness had begun in January 1952, when an isolated maculopapular eruption first appeared on the left arm. A month later a similar lesion appeared in the hip region. Subsequently maculopapular lesions appeared and disappeared all over the body except the face. Pruritus was severe, interfered with sleep and led to the first hospital admission on October 6, 1952. During this admission several antihistaminic drugs were given but provided no relief. Cortone® was administered beginning October 15th in the amount of 50 mg. at first and then 25 mg. every four hours thereafter. She was discharged as improved on October 22nd and the diagnoses made at that time were exfoliative dermatitis and neurodermatitis.

Following discharge from the hospital and withdrawal of cortone a marked recurrent reaction occurred. Skin lesions became markedly exfoliative and weeping, the patient was in constant distress, had fever of 101°F. and severe chills. Facial edema appeared and readmission to the hospital was necessary nine days after the patient had been discharged from the first admission.

Physical examination on October 31, 1952, revealed a maculopapular eruption, exaggerated in skin folds, covering the entire body. Some lesions were dry and red, others were crusted, and still others were weeping. Cervical nodes were moderately enlarged. Apart from the skin, no other body systems were abnormal.

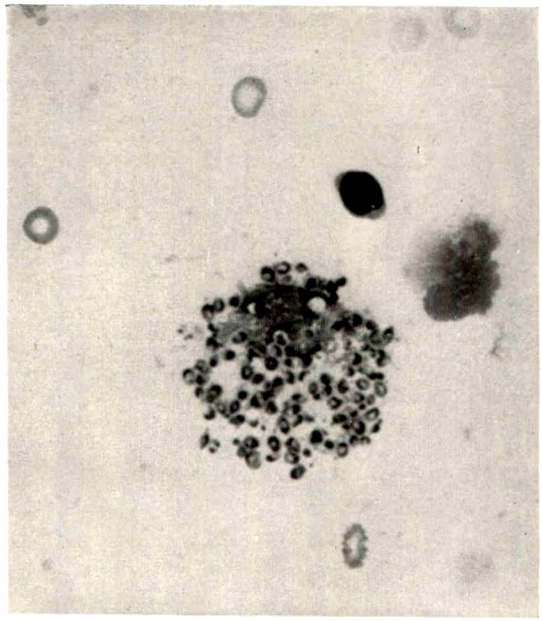


FIG. 26. Case III. Large cell from pleural exudate containing dozens of organisms; original magnification × 1000.

Laboratory examinations revealed the following: red blood cells 3.9 million; hemoglobin 11.5 gm.; white blood cells 10,300, 68 per cent polymorphonuclears, 9 per cent lymphocytes, 6 per cent monocytes and 17 per cent eosinophils. Serologic test for syphilis gave negative results. The urine contained 10 mg. of albumin and a few red cells. The chemical composition of the blood was within normal limits in all components studied.

The patient ran an intermittent fever ranging between 100°F. and 104°F. which was often associated with chills. No response to antibiotics was noted. On November 18, 1952, administration of ACTH was started; the temperature fell and the condition of the skin improved. At this time a heart murmur was noted in the apical and pulmonic areas, and diffuse abdominal tenderness was present. A small pleural effusion appeared. On November 21st H. capsulatum was identified in uncountable numbers in the pleural exudate (Fig. 26) and in the peripheral blood. (Figs. 27 and 28.) The patient was started on 2-hydroxystilbamidine. Her condition deteriorated and she died November 22nd.

* This case is presented through the kindness of Dr. H. Felson.

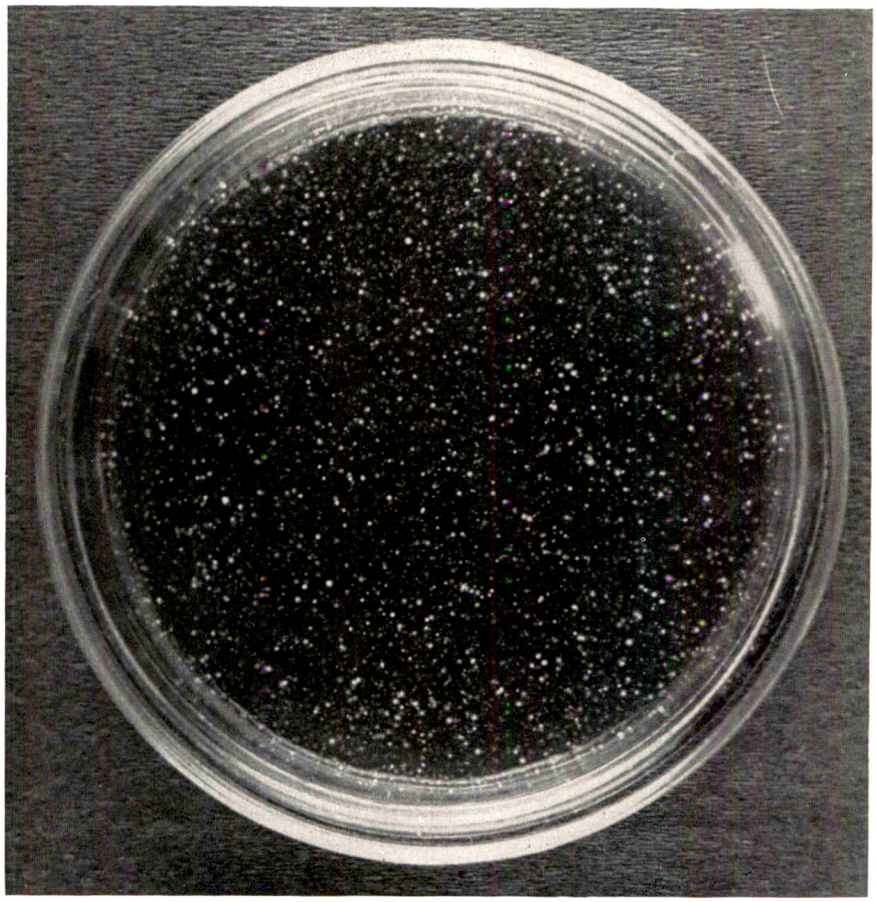
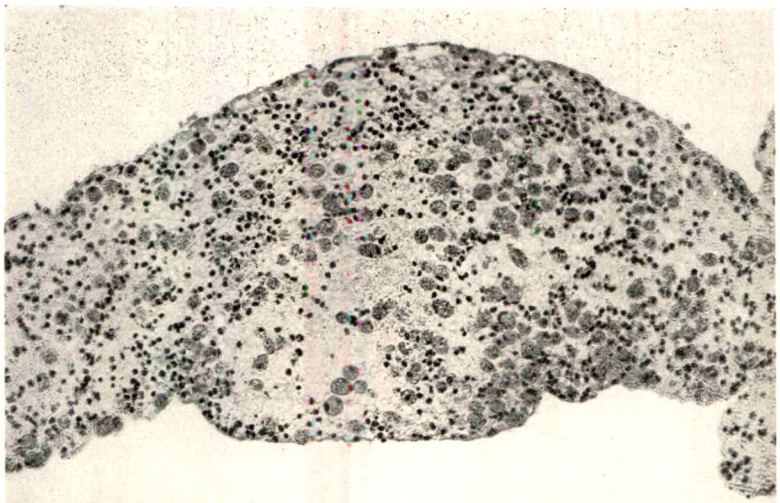


FIG. 27. Case III. H. capsulatum, 7000 colonies per cc. of blood, were counted. They were already visible after forty-eight hours; natural size of pour plate.

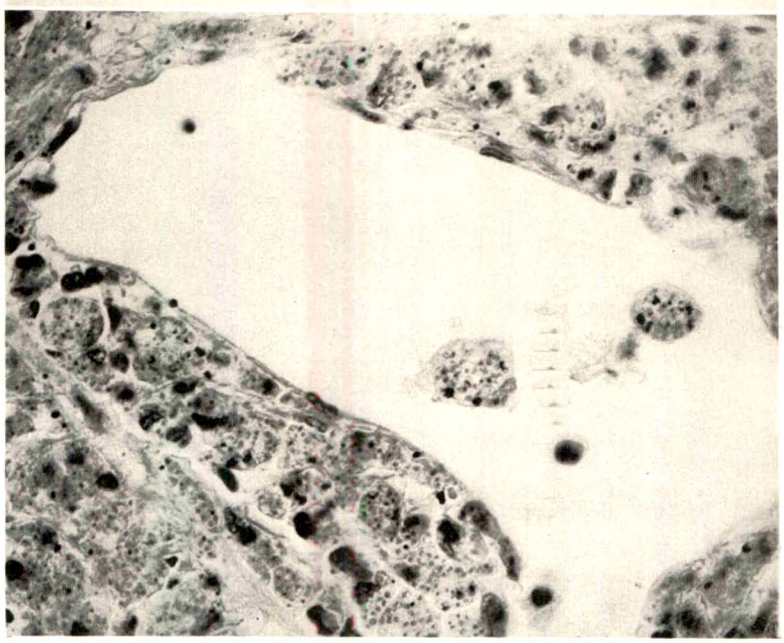
Autopsy. At autopsy, on external inspection, edema of the face, petechiae and scratch marks were prominent. (The serosal cavities all contained straw-colored fluid, the left pleura 800 cc., right pleura 500 cc., the pericardium 200 cc. and the peritoneum 300 cc.)

Organ weights: heart 280 gm., right lung 700 gm., left lung 600 gm., liver 2,700 gm., spleen 770 gm. and kidney 290 gm.

In the apex of the right lower lobe, there was a caseous focus of 10 by 7 mm. immediately below the pleura and the regional interlobular lymph nodes showed enlargement and caseation which was found also in a lymph node in the right inferior hilus. The rest of the thoracic lymph nodes were swollen but showed only spot-like foci of caseation. Tracheal nodules of 1 to 2 mm. were visualized and similar lesions were found in the left costal pleura. The lungs were solid throughout and only occasional miliary nodules on a diffusely brown background were recognized. Similarly, grayish nodules of 1 to 4 mm. were noted in the liver, the spleen, adrenals and kidneys. The gastrointestinal tract appeared normal on gross examination as was the heart, the internal genitalia, the thyroid and the pancreas. Microscopically, the primary lung focus contained a very large number of organisms and histiocytes which filled all alveoli or their remnants. There was complete loss of pulmonary architecture (but no caseation), and the overlying pleura showed fibrinous inflammation. The remainder of the lung showed innumerable histoplasma in phagocytes (Fig. 29), filling the alveoli, and less numerous interstitial (hematogenous) tubercle-like structures. Liver and spleen displayed unbelievable numbers of organisms with degenerative lesions of the parenchyma. Macrophages containing histoplasma were numerous in kidneys (Fig. 30), bone marrow (Fig. 31) and adrenals (Fig. 32), but were less numerous



28



29

FIG. 28. Case III. Blood clot taken from the heart with numerous parasitized phagocytes; original magnification × 160.

FIG. 29. Case III. Pulmonary vein; innumerable organisms in the phagocytes in the wall of the vessel and two cells in the lumen; original magnification × 650.

in skin, pancreas, thyroid and parathyroid. (Fig. 33.) The heart and the gastrointestinal tract were normal on microscopic examination. The lymph nodes throughout the body, in addition to the specifically described node formation related to the primary complex, were hyperplastic with enormous numbers of organisms in sinusoids and elsewhere.

Comment. This was a case of fulminating disease in a young adult in whom massive dissemination occurred while under treatment with adrenal cortical extract. The disease was so overwhelming and the organisms so numerous that they must have interfered with the function of organs in which they abounded by sheer mechanical displacement.

CASE IV.* E. B., a sixty-nine year old white man whose past history was not available, was admitted to Booth Hospital, Covington, Kentucky, June 4, 1951. In February, 1951, he had

* This case is presented through the kindness of Dr. D. Richfield.

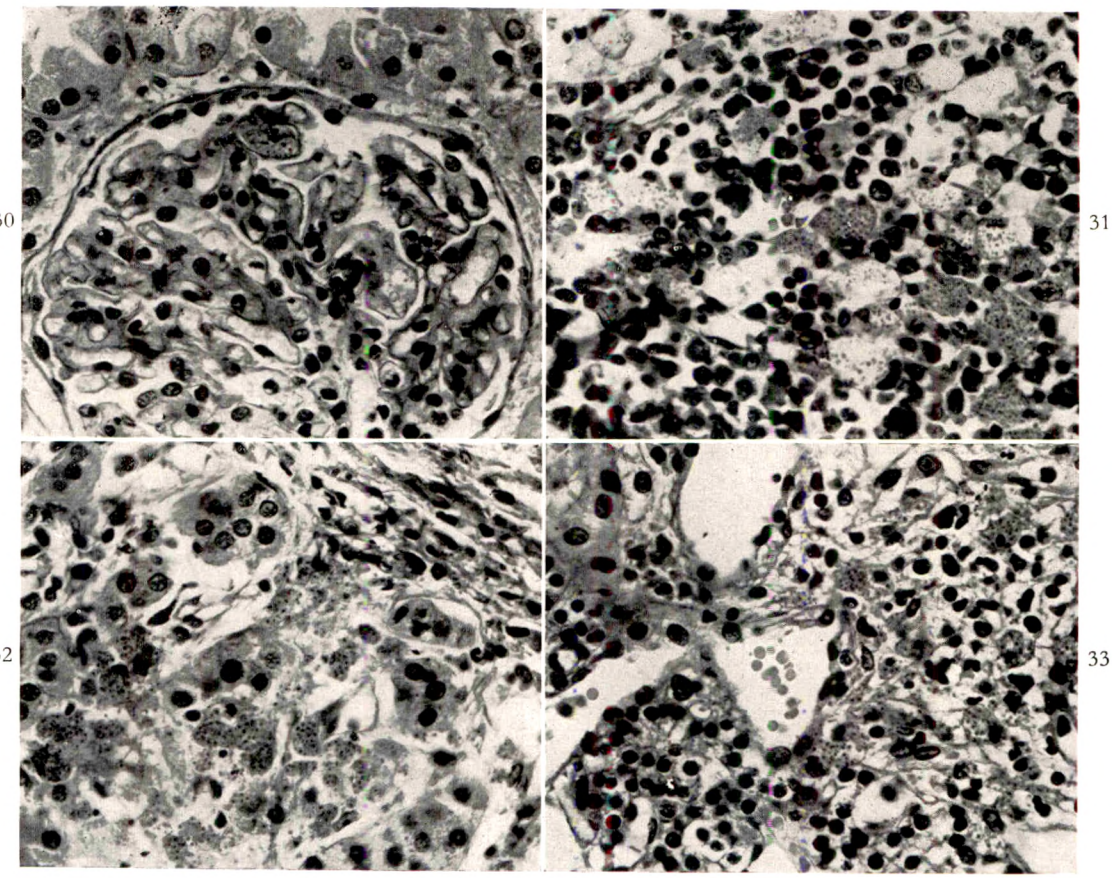


FIG. 30. Case III. In overwhelming invasion of the bloodstream all organs can become involved including the glomerular tufts; original magnification × 650.

FIG. 31. Case III. Bone marrow section; numerous organisms in swollen phagocytes; original magnification × 650.

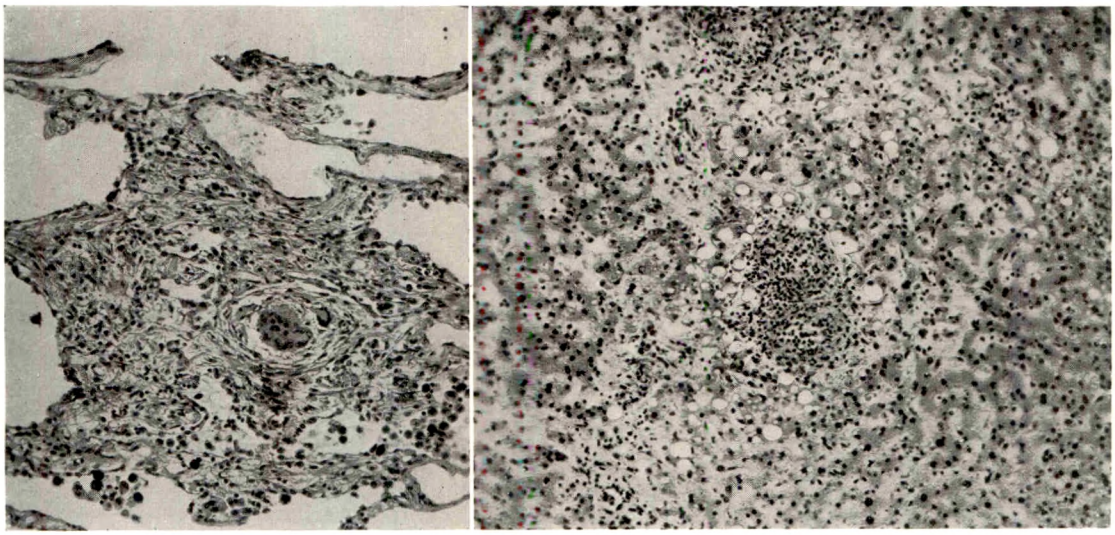
FIG. 32. Case III. Adrenal involvement; numerous intracellular organisms; original magnification × 650.

FIG. 33. Case III. Parathyroid involvement; original magnification × 650.

had an episode of cough and fever associated with positive roentgen signs of "pneumonia." He had been treated with penicillin; the response is not known. In April, 1951, roentgen examination of the gastrointestinal tract for unknown complaints was said to show "spastic colon and diverticula." Following this, without specific complaints, the patient became anorexic and gradually weakened. Shortly before admission to the hospital an acute decline occurred associated with cyanosis of the lips and mottling of the skin. In the belief that this represented "vasomotor collapse" administration of digitalis was begun on June 2, 1951, and he was also given lipoadrenal extract. Marked improvement occurred but was only temporary, and with return of somnolence he was hospitalized.

On physical examination he appeared chronically ill. He was dyspneic at bed rest; there was no cyanosis. The skin was sallow. The attending physician stated "it appears somewhat tan although he has not been in the sun." No lymphadenopathy was noted other than a left axillary node the size of a lima bean. The chest was mildly emphysematous. There was moderate non-tender hepatomegaly but no splenomegaly.

Laboratory data were as follows: The red blood count and hemoglobin within normal limits. The white blood cells numbered 5,000 to 7,000/cu. mm., but rose to 12,550/cu. mm. during the terminal stages. Initially, there was an eosinophilia of 21 per cent and a mild lymphocytosis; with cortisone treatment there was a gradual increase in neutrophils and stab cells, and a decrease in eosinophils and lympho-



34 35

FIG. 34. Case IV. Interstitial (hematogenous) fibrosing tubercle; original magnification × 160.

FIG. 35. Case IV. Submiliary necrosis and round cell infiltrates in liver; original magnification × 160.

cytes. Blood urea nitrogen was normal; bilirubin was 0.5 mg./100 cc.; total protein was 7.0 gm./100 cc. with the albumin comprising 2.8 gm. and the globulin 4.2 gm. A blood culture was sterile; urine was normal. Sputum was negative for acid-fast organisms. Roentgen examination of the chest demonstrated generalized miliary infiltration with left hilar enlargement with gradual but incomplete clearing. A needle biopsy of the liver was interpreted as showing miliary granulomatosis and fatty vacuolization; acid-fast stains gave negative results.

The patient was treated with antibiotics. Four days after admission a course of cortisone was begun; sudden improvement occurred on the eighth day of treatment and the patient went home when the drug was discontinued on the sixteenth day. There had been little febrile reaction at any time. Death occurred at home on July 12, 1951.

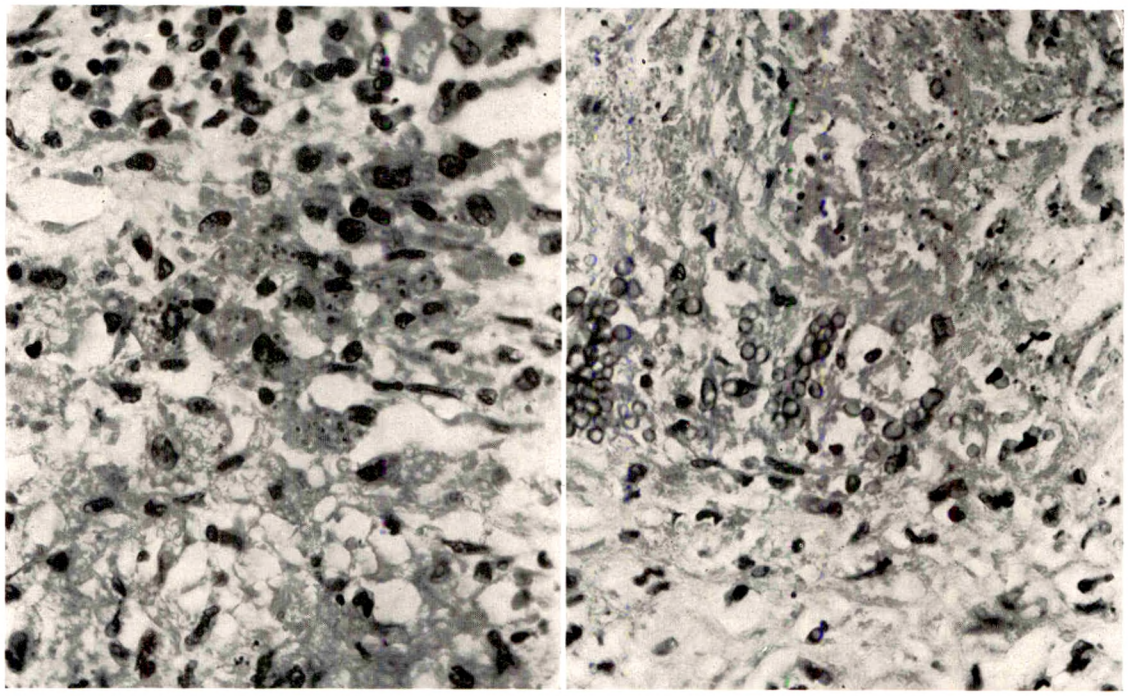
Autopsy. The body was that of a well nourished male of 165 cm. length and 75 kg. weight. The external inspection revealed no abnormality. In addition to extensive adhesions there were 100 cc. of straw colored fluid in each pleura. The right lung weighed 960 gm., the left lung 840 gm. A fine grayish granularity was more prominent in the upper lobes. The bronchial mediastinal lymph nodes were not remarkable. The liver was markedly enlarged (3,180 gm.) and showed miliary nodules. The spleen weighed 240 gm. The outstanding abnormality was found in the markedly enlarged adrenals (90 gm.) which were quite firm. Cortex and medulla were entirely replaced by reddish, grayish, yellowish mottled tissue. A small nodule was found in the pyramid of one kidney. Brain, heart and intestines were free of pertinent findings.

Microscopic Examination. In the lungs, a few interstitial tubercles were found which were very small and contained mostly epithelioid cells. (Fig. 34.) However, some foci had in addition numerous polymorphonuclear leukocytes. In some isolated spots H. capsulatum could be recognized definitely. All foci in the pulmonary parenchyma were of equal size.

Several lymph nodes of the bronchomediastinal region were examined and showed either scattered small abscess-like foci with polymorphonuclears and necrosis or the hyperplastic type of sinusitis with considerable swelling of the local histiocytes. Organisms in small number were recognized in some of the lymph nodes.

The spleen showed slight abnormality except for congestion but there were small circumscribed epithelioid tubercles between the otherwise normal cells.

In the liver were numerous foci, either small polymorphonuclear accumulations or necrosis plus polymorphonuclears. (Fig. 35.) No organisms were demonstrated in the liver lesion. Also



36A 36B

FIG. 36. Case IV. A and B, "small" and "giant" forms of H. capsulatum in the same adrenal; original magnification × 650; periodic acid Schiff stain.

present was an interstitial round cell myocarditis but no evidence of histoplasma was found. The outstanding observation was in the adrenals which showed complete caseation and some small typical organisms. In addition, there were a great number of yeast cells, of about 6 to 8 μ diameter, which stained quite differently from the usual histoplasma organism but in view of the demonstration of giant forms in tissue explants[180c] they can be accepted as representative for this species. (Fig. 36.)

In the bone marrow there were small foci of abscess-like metastases but no organisms were demonstrated.

Comment. This case represents a subacute or chronic form of histoplasmosis with hematogenous dissemination, manifested by foci in nearly all organs and diffuse caseation of the adrenals. Obviously, the patient could have overcome the hematogenous dissemination as shown by occasional scar formation in miliary nodules of the lung, if the destruction of the adrenal cortex had not produced an unsurmountable difficulty. This patient, like that discussed in Case III, was under treatment with cortisone.

CASE V.* B. S., a three year old white girl, had an episode of "acute pneumonia" in April, 1949, after three months' continuous residence on a farm in Kentucky. Prompt response to penicillin was noted although a blood count, taken twelve hours after institution of treatment, was said to be "inconsistent with pneumonia." Recovery from the acute episode apparently was complete in seventy-two hours.

However, subsequently the child was noted to be anorexic and irritable and became fatigued easily. Medical attention was sought approximately six months later. At this time, at the age of three and one-half years, the child was pale, had slight bilateral catarrhal otitis media and slight injection of the posterior pharynx. There were shotty submental and anterior cervical lymph nodes but no generalized lymphadenopathy was present. The percussion note was slightly impaired in the base of the chest on the right, and breath sounds were diminished in the same region. The liver edge was palpable 1 cm. below the right costal margin; the spleen was not felt. Hemoglobin was 72 per cent, white blood

* This case is presented through the kindness of Dr. Carl F. Wagner.

cells 14,400 per cu. mm. and the erythrocyte sedimentation rate was 17 mm. in one hour. Urinalysis gave normal results.

The patient was hospitalized for treatment and study. Tuberculin test gave negative results. A histoplasmin skin test gave positive results. Roentgen examination of the chest revealed enlargement of the right hilum and increased markings bilaterally consistent with pulmonary inflammatory reaction with hilar lymphadenomegaly. Although there was little febrile reaction, penicillin and chloromycetin were administered and the child was discharged after one week in the hospital.

During the hospital stay blood cultures were made for H. capsulatum and bone marrow was studied by smear and culture. Blood cultures were negative but positive cultures of H. capsulatum were obtained from two separate marrow aspirations. Only three of twenty-six plates which were inoculated gave positive results. Complement fixation studies gave positive results in a 1:20 dilution on several occasions and subsequently they gave negative results. The identification of the organism* was confirmed in two other laboratories.

Clinically, the child was well but pale and slow in developmental behavior for about six months after admission to the hospital. Only on supportive treatment of a dietary nature did she begin to improve, gain weight and become more active, and one year later was to all appearances a healthy child. The shadows in the roentgenograms gradually disappeared and questionable calcification was noted about eighteen months later.

Comment. This is an example of symptomatic non-fatal histoplasmosis which could easily have been missed had not careful mycologic studies been made. The symptoms were minimal and in no way indicated strong suspicion of histoplasmosis. They could easily have been overlooked, in which case later pulmonary calcification and positive skin sensitivity to histoplasmin would have had little definite significance. The finding of H. capsulatum in the bone marrow was almost incidental in this child, who had been discharged from the hospital as well fully two weeks before the diagnosis was established by the positive cultures.

CASE VI.† C. B., a twenty-three year old married university student, was admitted to the Jewish Hospital on April 20, 1953, because of increasing fatiguability, chills and fever, occasional arthralgia without swelling or redness of the joints and generalized malaise. The illness had begun three weeks prior to admission. The chills and fever had subsided with treatment by aureomycin but the other symptoms had persisted. A chest film prior to admission was reported to show "pulmonary congestion."

Past medical history was non-contributory. There had been no previous similar episodes. The patient had lived in the northeastern part of the United States all his life and had never been out of the country or visited the southern part of the United States or the West Coast.

Physical examination revealed a well developed and well nourished white man in no acute distress. Blood pressure was 130/75, pulse 96, temperature 99.6°F. Positive findings included a sharp, non-tender liver edge just palpable at the costal margin on deep inspiration. The spleen was also palpable but not tender. A few small, non-tender nodes were palpated in the anterior and posterior cervical chains and in the axillas.

The clinical impression on admission was fever of undetermined origin. Possible etiologic factors included virus pneumonia, infectious mononucleosis, histoplasmosis or tuberculosis, blood dyscrasia and brucellosis.

The patient's nine-day hospital course was characterized by an intermittent fever with diurnal elevation of 99.8°F. in the afternoon and a gradual decline to 97.8°F. by the next morning. Roentgen examination of the chest was reported to demonstrate enlargement of hilar shadows and some widening of the mediastinum. A battery of diagnostic procedures was undertaken. These included a complete blood count, sedimentation rate, heterophile agglutination, cephalin flocculation, thymol turbidity, alkaline phosphatase, total protein and A/G ratio, Van den Bergh test, prothrombin time, agglutinations for typhoid, paratyphoid, brucellosis, cold agglutinin titer, blastomycosis and histoplasmosis complement fixation test, stools for culture, ova and parasites, blood cultures, bone marrow aspiration, and tuberculin and histoplasmin skin tests. The histoplasmin skin test gave positive results, the sedimentation rate was 29 mm./hour and the prothrombin time was 60 per cent of normal; the remainder of the tests gave normal results except for one blood

* Initial isolation performed by Miss Dorothy Artis.

† This case is presented through the kindness of Dr. H. Felson.

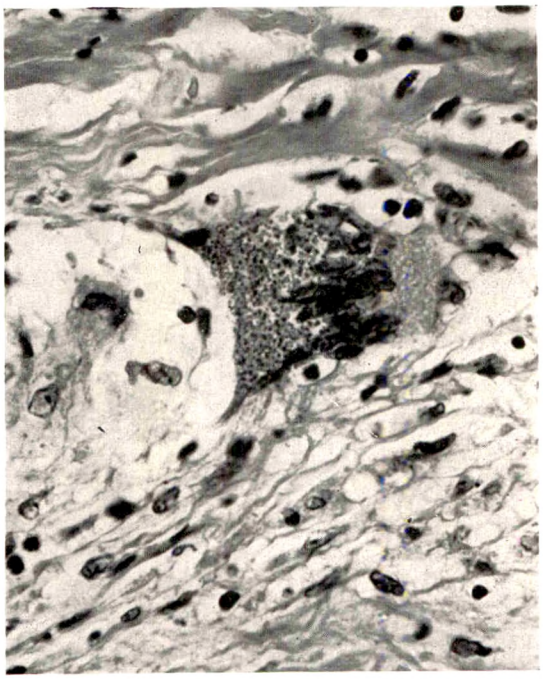


FIG. 37 Case VII. Epithelioid cells and multinucleated giant cells with numerous intracellular organisms; the greater part of the lymph node was calcified and partially ossified; original magnification × 800.

culture from which H. capsulatum was isolated the day after the patient left the hospital, improved but with his condition not diagnosed. At the time of discharge the intermittent fever persisted but the patient was subjectively better. Complement fixation was and still is (June, 1954) strongly positive, but fourteen months after the acute episode the patient is in excellent health.

Comment. This is another example of symptomatic non-fatal histoplasmosis in which the diagnosis would have been missed without mycologic cultures. Clinically, the patient had a mild to moderate fever of unknown origin from which recovery was taking place at a time when H. capsulatum were present in his blood stream.

CASE VII. B. J. S., an eleven year old white girl, was admitted to the hospital for surgical treatment of a patent ductus arteriosus. The clinical history and physical findings were typical for uncomplicated patency of the ductus arteriosus. The examining physician did elicit a history of recurrent "bronchitis" as a younger child. The anticipated treatment was carried out successfully and the patient is now clinically normal.

In preoperative roentgenograms of the chest, in addition to characteristic features of the cardiac malformation, a calcification was noted in intimate relationship to the dilated pulmonary artery. (Fig. 18.) Because of its accessibility to the area of surgery, the calcifying lymph node was removed by the surgeon at our request.

The lymph node measured approximately 12 by 8 by 6 mm. and was in its major part calcified. Microscopically (Fig. 37), numerous and fairly large foci of calcification were surrounded by areas with active granulomatous proliferation. There were small foci of histiocytic proliferation and a small number of multinucleated giant cells were seen. There was also caseation between the calcific masses. Occasional giant cells and histiocytes contained numerous organisms which were morphologically consistent with H. capsulatum. The culture from the lymph node and injection of the material into eight mice and reculture gave negative results, which probably reflects the fact that the organisms were not viable.

Comment. This patient illustrates the inapparent form of the disease for which positive proof in the past has been lacking or at best very scarce. It might be inferred, from evidence derived from the roentgenograms of intrathoracic calcification in this tuberculin-negative, histoplasmin-positive child, that the calcification and skin sensitivity were due to histoplasmosis, but in the absence of cultural or histologic proof this common combination of observations would have only statistical support.

SUMMARY

Histoplasmosis, once an "obscure" fungus disease, is recognized with increasing frequency, especially in the endemic area comprising Ohio, Kansas, Missouri, Indiana, Illinois, Kentucky and Iowa.

The disease is most frequently acquired by inhalation of spore-containing dust. The organism has been repeatedly recovered from soil and has given origin to local outbreaks in the form of small epidemics.

Hepatosplenomegaly and anemia with leukopenia occur in children. A picture very similar to any pulmonary disorder caused by tuberculosis is found in adults. Ulcerations in the oronasal region seem to be quite prevalent in adults.

The pathology of the disease is characterized by invasion of histiocytic elements by the

organisms. These multiply within the cells, which may become greatly enlarged. A productive and exudative response to the organism may be observed in many organs.

The skin test is an excellent epidemiologic tool and may also call attention to the possibility of histoplasmosis in clinical practice. However, only identification of the organism can be accepted as conclusive proof of disease, although a positive complement fixing test, especially with rising titer, contributes to a presumptive diagnosis.

The organism can be cultured successfully from many sites but bone marrow and blood are preferred. Sputum, bronchial washings and gastric lavage generally have to be injected into mice in order to avoid overgrowth by contaminating organisms. Liver and other biopsies may be decisive in establishing the diagnosis.

The mycologic diagnosis can be established by demonstration of the tuberculate spores produced at room temperature; the change of phase is an essential determinant in confirming the diagnosis. Sabouraud's glucose-agar or blood-agar are ideal media for isolation and identification of the organism.

Histoplasmosis has been found in several lower animals. In the laboratory, mice are ideal experimental subjects both for primary isolation and virulence studies.

No specific treatment is at present available; the value of surgical removal of lung lesions will have to undergo the scrutiny of time. If current estimates that 20 to 30 million people in the United States have histoplasma infections are correct, the magnitude of the problem becomes obvious. It is also apparent that histoplasmosis is a benign disease which only in exceptional cases becomes symptomatic and dangerous.

Seven hitherto unreported cases are described. In the endemic area many patients, upon initial examination, reveal active or healed lung lesions in the presence of positive histoplasmin and negative tuberculin reactions.

ADDENDUM

Since the preparation of this paper several other proved cases of histoplasmosis have been encountered. Among them was a fifty-five year old negro who had had cough and hemoptysis for one year associated with marked loss of weight. Studies for tuberculosis gave consistently negative results; material removed on bronchoscopy, by biopsy and by suction demonstrated carcinoma, but cultures and animal inoculation of the same material revealed Histoplasma capsulatum. Roentgen examination of the chest disclosed widespread densities in the upper portions of both lungs and definite cavitation. (Fig. 38.) This case is typical of the chronic progressive form in adults and is an example of histoplasmosis complicating other chronic lung disease, in this instance, carcinoma. The discovery of this and several other cases not included, further emphasizes that careful mycologic studies are necessary in the endemic area to permit recognition of the role played by H. capsulatum in morbidity and mortality.

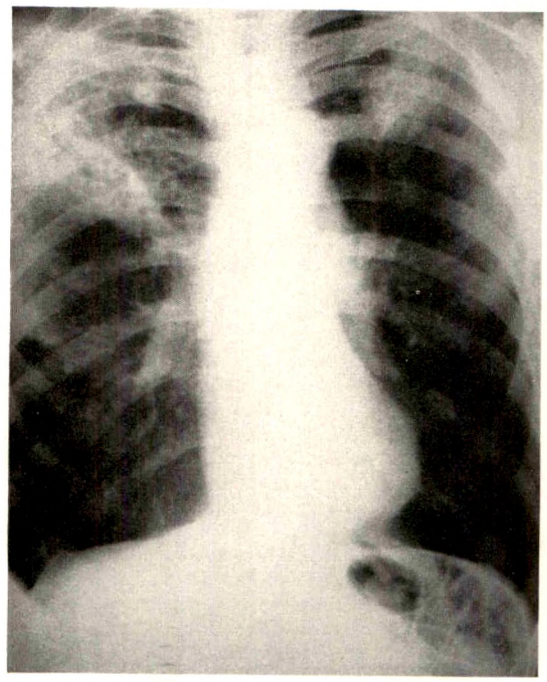


FIG. 38. Man, age 55; proved histoplasmosis, chronic progressive type; cough, hemoptysis and weight loss for one year; carcinoma of lung also present; H. capsulatum apparent from sputum and gastric lavage by culture.

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Seminars on Carbohydrate Metabolism

Spontaneous Hypoglycemia*

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IT has gradually became apparent to most physicians that periodic spontaneous hypoglycemia is the cause of significant and sometimes alarming symptoms in a relatively large number of patients. A widespread diagnostic awareness of the "hypoglycemic syndrome" as a cause of periodic symptoms has been finally induced as the result of a number of reviews[1–7] on the subject published during the past twenty years. Whereas formerly many such patients went undiagnosed because hypoglycemia was not considered, the diagnosis is now being made promiscuously and without the supporting evidence necessary to establish it.

This presentation has four major purposes: (1) to outline again the whole problem, incorporating recent additions to our knowledge and rejecting discredited concepts; (2) to demonstrate the clinical usefulness of a few simple but rigid criteria in establishing the specific etiologic diagnosis in more commonly encountered and confusing types of spontaneous hypoglycemia; (3) to discuss rational therapy for the various types, based upon present concepts of the abnormal mechanism which produces hypoglycemia in each instance; and (4) to attempt to prevent unnecessary pancreatic explorations and subtotal pancreatectomies in patients who exhibit no indication for such procedures.

DEFINITION OF SPONTANEOUS HYPOGLYCEMIA

Spontaneous hypoglycemia is not a disease. It is an indication of a derangement in the over-all utilization of carbohydrate in which glucose has been removed from the blood at a rate faster than it has been replenished, with a resultant depression of the blood sugar to an abnormally low level. As will be indicated later, many organic and functional lesions exist, any one of which is capable of increasing the rate of removal of glucose from the blood and/or of decreasing its rate of entry into the blood. Recognition of this fact constitutes the first step toward an intelligent appraisal of the mechanism by which hypoglycemic episodes are produced in any given patient.

By definition the term excludes hypoglycemia induced by the administration of exogenous insulin, but in the evaluation of the patient suffering from spontaneous hypoglycemia the possibility of a factitious cause (self-administration of insulin) must always be in mind. Also, by definition, the term excludes the concept of "relative hypoglycemia" which has crept into the literature to cause confusion in both diagnosis and management. Patients with vague emotional or gastrointestinal symptoms whose blood sugar levels, either when symptomatic or in the course of a glucose tolerance test, remain essentially normal are said to have "relative hypoglycemia."[8–10] It is implied that such individuals are unusually sensitive to minor depressions of blood sugar values. In all patients with unequivocal spontaneous hypoglycemia, low blood glucose values (below 55 mg. per cent by the Folin-Wu method,[11] or below 40 mg. per cent by the Somogyi-Nelson method[12]) can be demonstrated repeatedly. The conditions under which such diagnostic levels occur will aid greatly in disclosing the nature of the abnormality.

It should be clear now that spontaneous hypoglycemia and hyperinsulinism are not synonymous terms. The former is the generic term. It includes all clinical situations in which the blood sugar may fall to abnormally low levels spontaneously. The term hyperinsulinism

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is confined to those types of spontaneous hypoglycemia in which an absolute increase in the production of endogenous insulin is believed to occur.

SYMPTOMS OF HYPOGLYCEMIA

Unusual manifestations of hypoglycemia as well as their relation to the actual level of the blood sugar have often caused confusion in diagnosis. At opposite poles of the spectrum of symptoms are two distinct clinical pictures. The appearance of one pattern or the other, or of a combination of the two, is determined not only by the level of the blood sugar but also by the rate at which it has fallen and the duration of the hypoglycemia. If the rate of fall in blood sugar is rapid, the predominant early symptoms are those produced by compensatory hyperepinephrinemia. This mechanism represents an attempt to restore normal blood glucose levels by accelerating hepatic glycogenolysis.[13] The symptoms are those which are observed during a mild reaction after administration of too much soluble insulin, and consist of sweating, weakness, hunger, tachycardia and "inward trembling." Conversely, if the blood sugar falls slowly to low levels over a period of many hours, the manifestations are cerebral in type:[14] headache, visual disturbances, mental confusion, coma and convulsions. If the decrease in blood glucose is rapid, profound and persistent, the initial symptoms due to excessive circulating epinephrine merge with those of cerebral hypoglycemia.

When the blood sugar concentration remains low for most of the twenty-four-hour period, a situation which may occur in organic hyperinsulinism, the manifestations of hypoglycemia may be so bizarre as to be completely misleading.[6] Any aspect of the entire range of neurologic or psychiatric disorders may be mimicked, including sensory or motor loss of an extremity, hemiplegia, outbursts of temper, extreme depression or apparent catatonic schizophrenia. Despite the atypical nature of symptomatology in some individuals, it is of interest that the same complex of symptoms tends to recur in the same patient. In our experience periodicity of symptomatology represents a more important diagnostic hint of underlying hypoglycemia than the specific type of complaint which is offered. The repetitive nature of the complaint, occurring at fairly specific times during the twenty-four-hour period, should arouse suspicion. The demonstration of hypoglycemia during an attack, together with dramatic relief of symptoms with intravenously administered glucose, establishes only the non-specific diagnosis of spontaneous hypoglycemia. The specific etiologic diagnosis must now be established if rational and effective therapy is to be applied.

CLASSIFICATION OF SPONTANEOUS HYPOGLYCEMIA

It is beyond the intent of this presentation to attempt to discuss the body's complex physiologic mechanisms which, when properly integrated, result in normoglycemia. Mention of the more important tissues, organs and endocrine glands involved is sufficient to impress one with the multiplicity of possible aberrations which can upset the delicately balanced level of blood sugar. The list includes the central nervous system, the autonomic nervous system, the gastrointestinal tract, kidneys, liver, muscles, pancreas, adenohypophysis, adrenal cortex and medulla, and the thyroid.

Table 1 presents a classification of spontaneous hypoglycemia based upon etiologic factors. Some of the situations listed are associated only rarely with hypoglycemia, but are included for purposes of more complete classification. The more commonly encountered types will be discussed in detail.

TABLE 1

ETIOLOGIC CLASSIFICATION OF SPONTANEOUS HYPOGLYCEMIA

(Enlarged from original.[15])

1. Organic—recognizable anatomic lesion
   - A. Hyperinsulinism
     1. Pancreatic islet cell adenoma[7]
        - a. Single
        - b. Multiple
        - c. Aberrant
        - d. Associated with adenomas of other endocrine glands (parathyroid,[16] anterior pituitary[6,17])
        - e. Pancreatic islet cell "suspiciously malignant" adenoma (localized)
     2. Pancreatic islet cell carcinoma (with metastases)[5–7]
     3. Generalized hypertrophy and hyperplasia of the islets of Langerhans[18–20]
   - B. Hepatic disease
     1. Ascending infectious cholangiolitis[21]
     2. Toxic hepatitis[22–24]
     3. Diffuse carcinomatosis[25–28]
     4. Fatty degeneration[29] or "fatty metamorphosis"[30]
     5. Laennec's cirrhosis[31,32]
     6. Viral hepatitis[32,33]
     7. Chronic passive congestion in congestive heart failure[34]
     8. Glycogenosis (von Gierke's disease)[35,36]
   - C. Anterior pituitary hypofunction
     1. Destructive lesions (chromophobe tumors, craniopharyngiomas)[37–40]

TABLE I (*Continued*)

2. Atrophy and degeneration (Simmonds' disease)[41–44]
3. Surgical hypophysectomy[45]
4. "Pituitary myxedema"
   a. Thyroid hypofunction secondary to pituitary failure[46–48]
   b. Pituitary failure secondary to myxedema[49–52]
5. Severe inanition[53,54]
6. Postoperative hypoglycemia[55,56]

D. Adrenocortical hypofunction
1. Idiopathic cortical atrophy[57]
2. Destructive infectious granuloma[58]
3. Destructive neoplasm[59]
4. Congenital adrenal hyperplasia[60]

E. Fibromas and sarcomas[61]
F. Central nervous system lesions (hypothalamus or brain stem; interference with nervous control of blood sugar)[62–67]

II. Functional—no recognized anatomic lesion, but explainable on basis of unusual somatic function
A. Hyperinsulinism (imbalance of the autonomic nervous system); "hypoglycemic fatigue; nervous hypoglycemia; functional hypoglycemia, reactive hypoglycemia"[8,68–76]
B. Alimentary hyperinsulinism (rapid intestinal absorption)
1. After gastroenterostomy[77]
2. After partial or total gastric resection[77–82]

3. Hyperinsulinism of infancy (Staub-Traugott phenomenon)[51]
D. "Idiopathic spontaneous hypoglycemia of infancy"[51]
E. Renal glycosuria (severe degrees of low renal threshold for glucose)[83]
F. Lactation[84,85]
G. Severe continuous muscular work[86]

III. Factitious (surreptitious insulin administration)[87–90]

As an initial approach to placing a given hypoglycemic patient into his proper category etiologically, we have found it helpful to classify spontaneous hypoglycemia in still another way: the fasting hypoglycemias, the stimulative hypoglycemias, and those types in which both phenomena are demonstrable.

I. Fasting hypoglycemia: In this group the lowest level of blood sugar of the twenty-four-hour period is always obtained after the longest period without food. This usually means just before breakfast, providing a three-meal schedule is being followed during the day.

1. Hepatogenic hypoglycemia
2. Anterior pituitary insufficiency
3. Adrenal cortical insufficiency
4. Central nervous system lesions
5. Fibromas and sarcomas
6. Severe renal glycosuria

II. Stimulative hypoglycemia: This group exhibits consistently normal levels of the fasting blood sugar. It is two to four hours after the beginning of absorption of carbohydrate that hypoglycemia occurs. Measures designed to prevent the postprandial rise of blood sugar abolish the secondary hypoglycemic dip which follows it.

1. Functional hyperinsulinism
2. Alimentary functional hyperinsulinism
3. Hyperinsulinism of infancy

III. Combined fasting and stimulative hypoglycemia:

1. Organic hyperinsulinism
2. "Idiopathic spontaneous hypoglycemia of infancy"
3. Factitious (exogenous hyperinsulinism)

DETERMINATION OF ETIOLOGY IN THE INDIVIDUAL CASE

It will be clear from reviewing the etiologic classification that in most of the causes listed spontaneous hypoglycemia represents but one facet of an obvious clinical entity which is usually easily recognized. Thus one scans the patient first for clinical signs of adrenal or pituitary insufficiency, for central nervous system damage, for an abdominal scar which might indicate a gastric operation, for lactation or renal glycosuria, etc. Much less obvious is the rare case of self-administration of insulin. This possibility should come to mind when the patient is a nurse, physician, diabetic or a close relative of a diabetic who takes insulin. The suspicion is heightened when severe hypoglycemia occurs at unexpected times and without similarity of time pattern from day to day. The etiologic factors mentioned in this paragraph, however, represent the cause of symptomatic hypoglycemia is less than 15 per cent of the total number of cases encountered.

From the point of view of apparent difficulty in differential diagnosis, three causes of spontaneous hypoglycemia stand out and together they account for about 80 per cent of all cases. They are (1) functional hyperinsulinism, (2) organic hyperinsulinism and (3) hepatogenic hypoglycemia. Functional hyperinsulinism is the most common cause of symptomatic hypoglycemia and appears to be a psychosomatic disorder. The hypoglycemia, however, can be

controlled by rigid adherence to a high protein, low carbohydrate diet. Organic hyperinsulinism requires surgery. In this condition hypoglycemia is intensified by a diet low in carbohydrate and is only temporarily alleviated by one high in carbohydrate. Proper differential diagnosis of these two conditions results in selection of the proper patient for exploration of the pancreas, and avoids unnecessary subtotal pancreatectomies in those who had no indication for surgery in the first place.

Hepatogenic hypoglycemia may be acute and transient or chronic and persistent, depending upon the etiology of the diffuse hepatic lesion. The prognosis is dependent upon the etiology of the hepatic lesion. In most cases dietary control of the hypoglycemia (high protein, high carbohydrate diet with an extra feeding at bedtime) is all that can be offered. Occasionally, removal of the source of a chronic, active, ascending cholangiolitis (cholecystectomy) results in remarkable improvement in hepatic function with relief of fasting hypoglycemia.[21]

In attempting to differentiate these three important causes of spontaneous hypoglycemia, we have found the following guides to be of greatest value: (1) the history and clinical course of the disturbance; (2) the level of the fasting blood sugar: (*a*) on an average intake of carbohydrate, (*b*) upon restriction of carbohydrate or after a period of fasting; (3) the results of a glucose tolerance test performed after standard dietary preparation;[91] (4) the results of a battery of liver function tests.

Table II summarizes the usual distinguishing characteristics among the three types, and

TABLE II

Type of Hypoglycemia	Fasting Blood Sugar		Glucose Tolerance Curve	Liver Function	Clinical Course
	Standard Diet	CHO Restriction or 24 Hour Fast	After Standard Dietary Preparation		Progression and Time of Attacks
Functional hyperinsulinism	Normal	Normal*	Normal fasting blood sugar; sharp fall to hypoglycemic levels between 2nd and 4th hours	Normal	Not progressive in severity; more frequent under emotional or physical tension; relief by vacations, etc.; no prebreakfast attacks; attacks 2 to 4 hours after meals; no effect of skipped or late breakfast
Organic hyperinsulinism	Subnormal: usually below 50 mg. %	Subnormal: always below 40 mg. %; usually below 30 mg. %	Subnormal fasting blood sugar; low level curve;† sharp fall to very low levels between 2nd and 5th hours	Normal	Progressive in frequency and severity; prebreakfast attacks frequent from 2 A.M. to 8 A.M.; attacks 2 to 4 hours after meals; attacks precipitated by skipped or late meals, or exercise
Hepatogenic hypoglycemia	Subnormal: often below 50 mg. %	Subnormal: always below 40 mg. %; often below 30 mg. %	Subnormal fasting blood sugar; hyperglycemic plateau curve with glycosuria; gradual fall to hypoglycemic levels in 4 to 7 hours	Usually markedly diminished	Progressive in frequency and severity; prebreakfast the time of most frequent occurrence, 2 A.M. to 8 A.M.; daytime attacks rare unless precipitated by skipped meal; sometimes clinical evidence of hepatic disease

* During 3 days of severe carbohydrate restriction most normal people show fasting blood sugar levels of 50 to 60 mg. % (Somogyi).

† See text.

Figure 1 illustrates the characteristic oral glucose tolerance curve obtained in each.

CHARACTERIZATION AND MANAGEMENT OF SOME OF THE TYPICAL CLINICAL SYNDROMES WITH ILLUSTRATIVE CASES

Functional Hyperinsulinism

This is the only really common form of spontaneous hypoglycemia, and accounts for

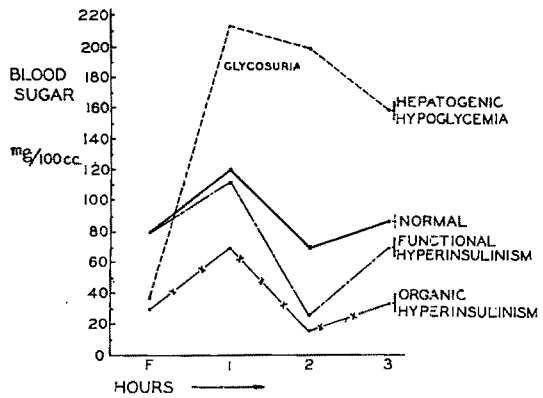


FIG. 1. Glucose tolerance curves in spontaneous hypoglycemia (three types).

about 70 per cent of all cases. Synonyms employed by various authors for this type of spontaneous hypoglycemia include: "hypoglycemic fatigue," "nervous hypoglycemia," "functional hypoglycemia" and "reactive hypoglycemia." The term functional hyperinsulinism seems preferable to us because it defines the most probable mechanism by which the hypoglycemia is produced.[15] All who have studied the problem agree that the disorder involves a disturbance in the finer regulation of the blood sugar by the vegetative nervous system.

Insulin-producing pancreatic islet cells secrete in response to a rising concentration of arterial blood sugar.[92–94] The influence of the autonomic nervous system upon this mechanism has received little study although vagal effects are probably important.[94,95] Until more information is available on this point, we choose to regard an increased vagal effect as increasing the sensitivity and responsiveness of the insulin-producing cells to a given rise of arterial blood sugar. Functional hyperinsulinism is thus considered to represent a hypersensitivity and an excessive responsiveness of histologically normal islet cells to the normal stimulus for insulin secretion. Under these circumstances a normal postprandial rise in blood sugar elicits an excessive secretion of insulin. The existence of concurrent emotional tension in such patients is beyond dispute. Demonstration that the postprandial secondary hypoglycemia exhibited by these patients is actually associated with hyperinsulinemia must await an accurate method for assaying circulating insulin.

The general physiologic and psychologic status of individuals subject to attacks of functional hyperinsulinism is interesting. They are intense, driving and intensely conscientious. They typify the so-called "vagotonic personality."[68,71] They are total hyper-responders, gastric hyperacidity and vasomotor instability being frequent concurrent manifestations of a general instability of the vegetative nervous system. Some authors have emphasized the obvious psychosomatic aspects of functional hyperinsulinism.[69,71,90] In many of these patients it is abundantly clear that after having established functional hyperinsulinism as a major cause of symptomatology, one is forced to make the additional diagnosis of an anxiety state. However, it is of practical importance to the patient that the hypoglycemia, which often produces his most troublesome symptoms, can be controlled by dietary means when environmental tensions persist.

Clinical Characteristics. 1. Relation of attacks to meals: The hypoglycemia is purely stimulative (postprandial) in type. It never occurs in the fasting state. Prebreakfast attacks do not occur, nor do symptoms develop during the morning when the patient omits breakfast. Symptoms occur only after meals and usually appear in two to four hours. The periodicity of symptoms in relation to time comprises the most helpful clue to the underlying disturbance.

2. Character of the symptoms: The manifestations of functional hyperinsulinism are essentially those of hyperadrenalinemia: weakness, hunger, "inward trembling," sweating and tachycardia. They are usually mild and transitory, often subsiding within fifteen or twenty minutes. Although early symptoms of cerebral hypoglycemia may supervene (diplopia, confusion, faintness, "black out spells"), it is a differential diagnostic point of great value that actual loss of consciousness or convulsions never occur in functional hyperinsulinism.

3. Progression of symptoms: In contrast to the intensification of symptoms with time observed in organic hyperinsulinism and hepatogenic

hypoglycemia, the manifestations of functional hyperinsulinism are not progressive. They tend to vary in proportion to the magnitude and persistence of environmental pressures.

4. The glucose tolerance test: The oral glucose tolerance curve is pathognomonic (Fig. 2) providing that standard dietary preparation is employed.[91] The fasting blood sugar is always normal, as is the absorptive rise. The secondary hypoglycemic phase, however, is rapid and profound. Unless levels below 40 mg. per cent (true blood sugar) can be demonstrated, the test is not regarded as diagnostic. It is imperative that blood samples be obtained at half-hour intervals after the second hour of the test for two additional hours. Almost invariably the patient's characteristic symptoms occur during the test, and the time of symptoms coincides with the time that hypoglycemia occurred.

Illustrative Case. Patient N. P., a twenty-eight year old single, female, art teacher, was admitted on April 16, 1954, complaining of recurrent spells of sleepiness and epigastric pain of three years' duration. These occurred usually late in the morning or afternoon, but occasionally about 9 P.M. They were accompanied by sweating, "inward tremor," weakness and a sense of loss of contact with her surroundings. Palpitation was noted during some episodes, and blurred vision during others. Attacks usually lasted fifteen to twenty minutes and, although she had "almost blacked out" several times, actual loss of consciousness had never occurred. The patient recognized a definite correlation between the frequency of attacks and physical or emotional exhaustion resulting from excessive hours of work. Benzedrine had afforded no relief of symptoms. For a three-month period, however, she had noted relative freedom from spells while following a self-devised high protein diet divided into six feedings daily. Symptoms recurred promptly after returning to a normal three-meal diet. Except for these episodes she considered herself to be entirely well.

On physical examination the patient's general health appeared to be excellent. Her personality pattern was an admixture of intense, driving energy and effusive, pleasant and outwardly calm demeanor. She blushed frequently and large areas of vasodilatation on neck and chest remained after facial flushing disappeared. Dermatographia was demonstrable. The palms were cool and clammy. The remainder of the examination revealed nothing abnormal.

After standard dietary preparation for three days, the glucose tolerance curve was as follows (Fig. 2): fasting, 59 mg. per cent (Somogyi); one hour, 148 mg. per cent; two hours, 92 mg. per cent; two and one-half hours, 53 mg. per cent; three hours, 22 mg. per cent; three and one-half

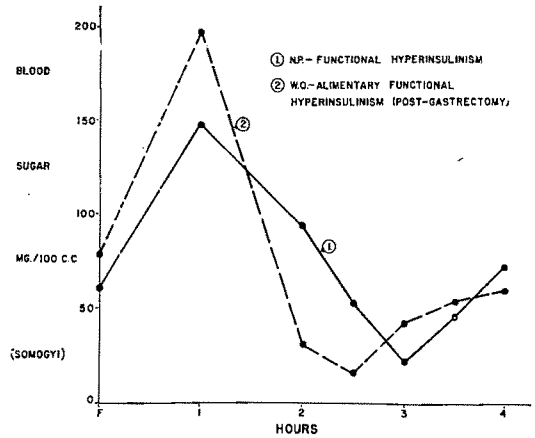


FIG. 2. Glucose tolerance curves in functional hyperinsulinism and in alimentary functional hyperinsulinism.

hours, 46 mg. per cent; four hours, 74 mg. per cent. Following the two and one-half hour sample the patient noted the onset of weakness and shakiness, which partially subsided after the third hour but persisted to some extent until she had lunch after completion of the test.

A diagnosis of functional hyperinsulinism was apparent, but in order to rule out the unlikely possibility that symptoms were caused by a functioning insuloma, the patient was fed a diet of 50 gm. protein, 50 gm. carbohydrate and 1,200 calories for three days. On this regimen she remained asymptomatic, and daily fasting blood sugar values were: before starting the diet, 64 mg. per cent; after twenty-four hours, 44 mg. per cent; after forty-eight hours, 55 mg. per cent; after seventy-two hours, 64 mg. per cent. Adherence to a therapeutic diet containing 130 gm. of protein, 75 gm. of carbohydrate and maintenance calories resulted in prompt and sustained relief of all major symptoms.

Management. Curtailment of excessive postprandial production of insulin by minimizing the postprandial rise in blood sugar eliminates the secondary hypoglycemia. Early methods of treatment helped to establish this concept. Low carbohydrate, high fat diets with frequent feedings[96] or administration of ten units of soluble insulin thirty minutes before each

meal[97] were partially effective but unwieldy and impracticable.

In 1936 experimental and clinical data were reported[98] indicating the singular advantage of a diet high in protein in the management of these patients. In the nineteen years that have ensued, the high protein, low carbohydrate diet has proved in our experience to be the most effective form of therapy for functional hyperinsulinism. This has been confirmed both clinically[68,70,72,73,78,89] and experimentally.[99] Depending upon the tolerance of the individual for protein, we prescribe diets containing 120 to 140 gm. of protein, 75 to 100 gm. of carbohydrate and maintenance calories. This is given as three meals, the protein and carbohydrate allotments being divided as equally among the meals as is practicable.[100]

Portis[8] has attempted to circumvent the chief objection to this dietary regimen, the rigid carbohydrate restriction, by eliminating only foods containing rapidly absorbed "free sugar" and relying on atropine to block the vagotonia. However the amount of atropine required (gr. $\frac{1}{200}$ to $\frac{1}{75}$ three or four times a day) often causes side-effects as troublesome as those of hypoglycemia. Secondly, we have demonstrated that the postprandial rise of blood sugar following ingestion of isoglucogenic quantities of polysaccharides is essentially the same as that produced by glucose.[101]

Despite older reports of its occasional efficacy[102,103] pancreatic resection as a therapeutic approach in functional hyperinsulinism is wrong. The only excuse for such surgery today is the uncommendable one that an erroneous diagnosis of organic hyperinsulinism was made preoperatively.

Alimentary Functional Hyperinsulinism

Recognition of this type of spontaneous hypoglycemia should present no difficulty, since it is seen only as a sequela of gastric surgery. Aside from its clinical importance, however, it warrants discussion in some detail because it clarifies several points concerning the mechanism which produces the stimulative type of spontaneous hypoglycemia.

A final figure on the incidence of clinically significant postprandial hypoglycemia after gastric surgery is difficult to establish. Evensen[77] reported its occurrence in thirteen of thirty-seven patients following gastroenterostomy, and in ten of ninety-seven patients after gastrectomy. Fifteen of Zollinger's twenty-five gastrectomized patients with postprandial gastrointestinal symptoms had symptomatic hypoglycemia, which he believes occurs more commonly than is generally realized.[80] Gilbert and Dunlop[81] established its presence in seventeen of forty-five consecutive partial gastrectomies; and over a three-year period Adlersberg[104] saw fourteen patients with the postgastrectomy hypoglycemic syndrome. Although Welbourn[82] found significant postprandial hypoglycemia in only a few of his 300 subtotally gastrectomized patients, Capper reported an incidence of 5.2 per cent in a series of 600 such patients.[82] The actual frequency of this type of symptomatic hypoglycemia is further obscured by the fact that some patients are aware of such symptoms for but a short time following surgery, despite persistence of a profound hypoglycemic dip during subsequent glucose tolerance tests.[77,82] Nevertheless, review of the over-all data creates the impression that this type of spontaneous hypoglycemia is next in importance numerically to functional hyperinsulinism. It is likely that its incidence will increase in parallel with the increasing number of gastric operations being performed.

The role of the pyloric valve in the regulation of carbohydrate metabolism may not be appreciated until one observes the result of a by-passing operation. In the normal person ingested carbohydrate is released through the pylorus in spurts. Absorption from the small intestine extends over a relatively prolonged period. In a gastrectomized individual, however, food may pour uninterruptedly through the stoma and suddenly cover a large surface of small bowel. Absorption of glucose is then excessively rapid and results in an abnormally high initial elevation of blood sugar. This is seen best in the course of an oral glucose tolerance test. (Fig. 2.) From a normal fasting blood sugar value a rapid rise to hyperglycemic levels (usually between 200 and 300 mg. per cent) occurs within one hour. Since the patient has normal islet cell function, the initial hyperglycemic phase constitutes an excessive stimulus for insulin secretion which in turn produces a very rapid fall of the blood sugar to hypoglycemic levels. Evensen[77] and others[79] have demonstrated that the same phenomenon occurs in normal persons to whom glucose is administered by intraduodenal tube.

In Figure 2 this type of response is contrasted

with that seen in the usual variety of functional hyperinsulinism. Although the postprandial hypoglycemia which occurs in both groups is presumably the result of a sudden and abnormally large release of insulin into the blood, a sharp distinction must be made between the mechanisms involved in the two situations. Functional hyperinsulinism represents an abnormal response of functionally hyper-reactive islets to the normal insulogenic stimulus, since the initial elevation of the blood sugar is within normal limits. On the other hand, alimentary functional hyperinsulinism represents the normal response of normally sensitive pancreatic islets to an abnormal insulogenic stimulus, namely, a very high initial elevation of the blood sugar level. This, then, represents a functional pancreatic response of a different type.

The clinical manifestations of alimentary functional hyperinsulinism should not be confused with the well known "dumping syndrome," which is also a sequela of gastroenterostomy or gastrectomy. When postprandial hyperosmolar jejunal contents draw fluid rapidly into the lumen and distend it,[105] symptoms of epigastric fullness, nausea, sweating, weakness and palpitation appear within a half hour after eating and subside in less than an hour.[105,106] These symptoms are not due to hypoglycemia. A period of about an hour then elapses before the onset of hypoglycemia. Hypoglycemic symptoms appear abruptly within one and a half to three hours after meals and are primarily those of hyperadrenalinemia. Occasionally, evidence of severe cerebral hypoglycemia (diplopia, headache and mental confusion) supervene; and rare instances of loss of consciousness and even convulsions have been reported.[80] Not all patients with symptoms of the "dumping syndrome" have hypoglycemic episodes.

Since this is a pure type of stimulative hypoglycemia, the fasting blood sugar is always entirely normal and prebreakfast attacks do not occur.

Illustrative Case. Patient W. O., a fifty year old white man, was admitted to University Hospital on January 26, 1954. Two years previously subtotal gastrectomy had been performed for bleeding gastric ulcer. One month postoperatively he had noted the onset of attacks of "trembly weakness" associated with sweating, dyspnea and tachycardia, which usually appeared about three hours after breakfast, but sometimes occurred also in mid-afternoon. Severe symptoms lasted but five to ten minutes, and subsided even more quickly if he ate something at the onset of an episode. Attacks had increased in frequency but not in intensity for five months prior to admission. He had never lost consciousness during a spell. Despite a good appetite he had lost 6 pounds in four months.

The patient appeared chronically ill and was malnourished. Height was 71 inches, weight 114 pounds, pulse 80 and blood pressure 110/78. Other positive findings were a transverse epigastric surgical scar; a non-tender and smooth liver edge which descended 10 cm. below the right costal margin on deep inspiration; and a palpable splenic tip 4 cm. below the left rib margin on deep inspiration.

Complete blood count and routine urinalysis were normal. Hepatogram revealed the following values: total serum bilirubin, 0.2 mg. per cent; gamma globulin, 8.2 units; thymol turbidity, 8.9 units; cephalin cholesterol flocculation, 3-plus after twenty-four hours and 4-plus after forty-eight hours; bromsulfalein retention, 1.0 per cent forty-five minutes after injection of dye; total serum cholesterol, 205 mg. per cent; total serum proteins, 6.8 per cent, with albumin, 4.5 per cent and globulin, 2.3 per cent. Barium swallow disclosed a normally functioning postgastrectomy gastroenterostomy stoma, with no evidence of obstruction or marginal ulceration.

After standard dietary preparation[91] for three days, the patient yielded the following glucose tolerance curve (Fig. 2): fasting, 77 mg. per cent (Somogyi); one hour, 194 mg. per cent; two hours, 32 mg. per cent; two and one-half hours, 16 mg. per cent; three hours, 41 mg. per cent; three and one-half hours, 54 mg. per cent; four hours, 60 mg. per cent. Just before the two-hour sample was drawn the patient noted the abrupt onset of weakness, inward trembling and sweating, with attenuation of these symptoms in about fifteen minutes. The diagnosis of alimentary functional hyperinsulinism was thus confirmed. Postprandial symptoms were abolished within three days by a diet containing 120 gm. of protein, 125 gm. of carbohydrate and maintenance calories.

Management. A medical program designed to prevent abnormally rapid intestinal absorption of glucose eliminates the initial hyperglycemia and, consequently, the secondary hyperinsulinism and hypoglycemia. Excellent results have

been obtained with the same high protein, low carbohydrate dietary regimen which is used in functional hyperinsulinism. Symptoms are well controlled on a three-feeding schedule, although some postgastrectomy patients prefer a six-meal regimen.

Organic Hyperinsulinism

The important differential diagnostic criteria which separate organic hyperinsulinism from other forms of spontaneous hypoglycemia have been outlined. (Table II.) However, a number of additional points in relation to this disease require discussion.

McQuarrie has recently delineated a syndrome which he has temporarily named "idiopathic spontaneous hypoglycemia of infancy."[61] (Table I.) This is discussed in detail later in the present paper. For integrative purposes, however, brief reference to it must be made at this point. This disease is characterized by severe fasting hypoglycemia without a demonstrable organic lesion. It occurs in infancy or early childhood and subsides spontaneously as the child grows older. In the active phase of this disturbance the clinical and laboratory findings are indistinguishable from those observed in organic hyperinsulinism. Thus it is now necessary to consider seriously the age of the patient before advising pancreatic exploration. Furthermore, since pancreatic islet cell tumors do occur below the age of five years (although this is very rare), a clear-cut differential diagnosis in this age group may be impossible by present testing methods.

Nature of the Lesion. In Howard's compilation of 264 hypoglycemic patients upon whom pancreatic surgery had been performed,[7] five were children below the age of five years. Two of them harbored an adenoma, while normal islet tissue was present in three. Critical review of the data in sixteen adults whose subtotally resected glands were thought to contain hyperplastic or hypertrophic islets, suggests that most of these cases had been instances of functional hyperinsulinism and that the rest probably harbored an undiscovered insuloma postoperatively. It is our opinion and that of others[89,90] that organic hyperinsulinism due to hyperplastic islet tissue is indeed rare in the adult.

About 9 per cent of functioning islet cell tumors are malignant, the presence of metastases being required for a diagnosis of malignancy.[5–7] No case of islet cell carcinoma has been reported below the age of eighteen.[7] Thus it would appear that in adults about 90 per cent of cases of organic hyperinsulinism are due to the presence of one or more benign, functioning islet cell adenomas. It is of some interest that over 80 per cent of islet cell adenomas (on the basis of autopsy findings) are devoid of function.[5,107]

Useful Procedures in Suspicious or Borderline Cases. As has been emphasized, a diagnosis of organic hyperinsulinism cannot be made unless unequivocal fasting hypoglycemia can be demonstrated repeatedly. In most cases this phenomenon is easily demonstrable even when the patient is ingesting a diet abundant in carbohydrate. In milder cases, however, severe restriction of carbohydrate may be necessary to prove the existence of fasting hypoglycemia. Wilder[68] advises a thirty-hour fasting period. When severe symptoms of hypoglycemia develop, requiring administration of glucose, the blood sugar level is expected to be 40 mg. per cent or less (Folin-Wu).

As the initial approach to suspicious cases we employ what we have termed a "provocative diet" for a period of three days. This diet contains 50 gm. of carbohydrate, 50 gm. of protein, and a total of 1,200 calories. The patient is hospitalized during this period and daily fasting blood sugar values are determined. If an insuloma is present the diagnosis is abundantly clear before forty-eight hours have elapsed. When diagnostic hypoglycemia has not become evident after three days on the provocative diet, the patient is fasted on the fourth day. This is followed by two hours of vigorous exercise on a stationary bicycle on the morning of the fifth day, the patient still fasting. When we have failed to demonstrate fasting hypoglycemia with the three-day provocative diet, we have failed also during the subsequent procedures. We believe, however, that the entire procedure must be carried out if one is to be certain that organic hyperinsulinism does not exist. The importance of this test or a similar one[68,108] in distinguishing functional hyperinsulinism from organic hyperinsulinism cannot be overemphasized.

The importance of the blood sugar lowering effect of exercise as a helpful diagnostic procedure in organic hyperinsulinism has been emphasized by Ford.[109] He observed that a previously symptomatic patient may have no attacks, and may even tolerate a day or more of fasting, during the tranquillity of hospital

bed rest. Prolonged delay in diagnosis, ranging from months[110] to years,[89] has been reported in patients with suspected insuloma, in which fasting blood sugar values remained above 50 mg. per cent during starvation in the hospital but in whom superimposed exercise was not employed. Nevertheless, in early or mild cases diagnostic criteria required for operation may not be demonstrable until further progression of the disease has occurred. However, if rigid diagnostic criteria are relaxed, a large number of patients who harbor no insuloma will be operated upon unnecessarily; and many of them will lose a large part of their pancreas.

Although the glucose tolerance curve is not the most important diagnostic test in organic hyperinsulinism, its value in differentiating this type of spontaneous hypoglycemia from others has been deprecated unduly. Some authors[6,20,88] seem mystified by responses which vary from low flat curves to high plateau curves in different patients and even in the same patient. We[111] have already demonstrated in insuloma patients the important alterations that can be produced in the glucose tolerance curve by changes in the antecedent diet; but another significant factor exists. Prolonged and continuous insulin secretion from islet cell tumors appears to suppress function of the normal islets.[112] The latter, however, can be restimulated to normal responsiveness by a few days of high carbohydrate feeding. In the most severe cases of organic hyperinsulinism in which normal islet function is maximally depressed, it may require a week of very high carbohydrate feeding (500 gm. a day or more) to restore normal responsiveness. The typical glucose tolerance curve can then be obtained. We have demonstrated this to be the responsible mechanism in a study of chronic exogenous hyperinsulinism in dogs[112] as well as in an insuloma patient given alloxan, the result of which was destruction of beta cells of the normal islets without any influence upon the insulin-secreting tumor.[113]

The mechanism is of more than academic interest since in hepatogenic hypoglycemia the plateau curve cannot be depressed by high carbohydrate feeding. A hypoglycemic fasting blood sugar, followed by a high plateau curve in the presence of normal liver function, does not require an attempt to produce the characteristic curve of organic hyperinsulinism by excessively high carbohydrate feeding. On the other hand, depression of the same type of curve by high carbohydrate feeding in the presence of evidence of hepatic impairment suggests that the cause of the hypoglycemia is pancreatic in origin.

Illustrative Cases. The two brief case reports which follow illustrate some of the points discussed:

CASE I. For two years prior to admission, fifty-one year old Mr. H. B. had noted progressive frequency and severity of prebreakfast mental confusion and late afternoon attacks of weakness, tremor, sweating and disorientation. He had had one convulsion before breakfast, and once had to be aroused in the morning by administration of glucose intravenously. At 4 A.M. of the first day of admission to University Hospital, he had a severe convulsion during which he sustained a fracture-dislocation of the left humerus. The fasting blood sugar drawn four hours later was 92 mg. per cent (Somogyi). That evening a closed reduction of the dislocation was performed under intravenous pentothal® anesthesia. On the standard preparatory diet, which contains 300 gm. of carbohydrate, fasting blood sugar values for the next three days were 46, 67 and 71 mg. per cent. The following day a glucose tolerance test revealed: fasting, 53 mg. per cent; one hour, 111 mg. per cent; two hours, 84 mg. per cent; two and one-half hours, 82 mg. per cent; three hours, 70 mg. per cent; three and one-half hours, 50 mg. per cent; four hours, 51 mg. per cent; four and one-half hours, 41 mg. per cent; five hours, 40 mg. per cent. The next morning the fasting blood sugar was 31 mg. per cent. Lunch was withheld to induce a short period of fasting. At 2 P.M. the blood sugar level was 33 mg. per cent and at 4 P.M. it was 27 mg. per cent. The patient exhibited obvious mental confusion, sweating and tachycardia. Orange juice relieved these symptoms promptly.

The next three daily fasting blood sugar values, while the patient continued to eat the standard preparatory diet, were 31, 36 and 38 mg. per cent. All tests of liver function gave normal values. The diagnosis of organic hyperinsulinism was now established. It is likely that failure to obtain diagnostic values during the first three days in the hospital was due to temporary alleviation of fasting hypoglycemia by the pituitary-adrenal response to the fracture.

Aqueous ACTH (40 units intramuscularly three times a day) was begun in preparation for operation. Fasting blood sugar values for the next three days were 118, 109 and 111 mg. per

cent. A benign insuloma was excised from the tail of the pancreas. The patient is now well.

CASE II. When Mr. A. H., a thirty-five year old white male, was admitted to University Hospital on September 7, 1952, he stated that unless he ate two sandwiches and drank two glasses of milk just before retiring, he did not wake up in the morning. He had been in entirely good health until May, 1950, when he first noted disorientation and amnesia upon arising after sleeping late on Sunday mornings. At the onset, breakfast quickly restored normal mental lucidity. Over the following few weeks, however, the frequency and severity of prebreakfast mental confusion increased until episodes were occurring daily and persisted for an hour or more after breakfast. In another hospital, five fasting blood sugar values (Folin-Wu) ranged from 36 to 49 mg. per cent and one was 63 mg. per cent. It was noted that each morning the patient was confused, and on one of these occasions symptoms disappeared within five minutes after glucose was administered intravenously. A diagnosis of functioning islet cell adenoma was made and on July 29, 1950, the pancreas was explored. When no tumor was found, splenectomy and an estimated 80 per cent pancreatectomy were performed. Diagnosis on the excised tissue was islet cell hyperplasia. (Subsequently, Dr. C. V. Weller, University Hospital, reviewed sections of this material and reported similarly: "Increased numbers of islets of Langerhans with increase in size of some of them. Moderate hyperplasia of islet tissue").

Within a week postoperatively, symptomatic prebreakfast hypoglycemia reappeared but was less severe than before the operation. In January, 1952, a severe attack of mental confusion occurred just before lunch. This responded slowly to ingested sugar and to intravenous glucose. Thereafter, frequent afternoon episodes accompanied by sweating, weakness and tachycardia appeared about two hours after lunch. One afternoon in February, 1952, he was found unconscious in a park. He responded to an intravenous injection of glucose. No further loss of consciousness occurred, but progressive frequency of prebreakfast, early afternoon and late afternoon attacks led to extreme introspection regarding his ultimate prognosis. He entered University Hospital for further evaluation on September 7, 1952.

Physical examination revealed no abnormalities other than moderate obesity.

On the standard preparatory diet for three days, with an additional feeding of 30 gm. of carbohydrate at bedtime, fasting blood sugar levels were 40 and 30 mg. per cent (Somogyi), and the glucose tolerance test gave the following values: fasting, 31 mg. per cent; one hour, 117 mg. per cent; two hours, 205 mg. per cent; two and one-half hours, 181 mg. per cent; three hours, 137 mg. per cent; three and one-half hours, 77 mg. per cent; four hours, 55 mg. per cent; four and one-half hours, 27 mg. per cent; five hours, 36 mg. per cent. It was concluded that the patient still harbored a functioning insuloma and that exploration of the pancreatic remnant was indicated.

For three days preoperatively the patient was given 40 units of aqueous ACTH intramuscularly every eight hours. On September 15, 1952, the day of surgery, the fasting blood sugar was 73 mg. per cent. At operation it appeared at first that the tail and most of the body of the pancreas had, in fact, been removed. However, careful exploration disclosed that most of the body remained. On the surface of the body of the pancreas a reddish purple nodule 2 cm. in diameter was observed. The nodule was excised, but when the frozen section suggested possible malignancy because of invasion of the capsule, the remaining portion of the body of the pancreas was resected. This procedure proved to have been unnecessary, since the final histologic diagnosis was benign islet cell adenoma.

Moderate elevation of the fasting blood sugar persisted until ACTH was withdrawn completely on the fifth postoperative day. Carbohydrate metabolism has continued to be normal to date (three and one-half years).

Management. When the diagnostic criteria have been satisfied, prompt surgical removal of the abnormal islet cell tissue is mandatory. It arrests progression of a disease which can eventuate in (1) permanent central nervous system damage,[6,65,114,115] (2) a prolonged and irreversible episode of hypoglycemic coma, or (3) increasing obesity which complicates future surgery. The opinion that delay of surgery might permit transformation of a benign adenoma to a malignancy is no longer held.[7]

For two reasons the patient should be prepared for surgery with ACTH or hydrocortisone. The primary purpose is to elevate the blood sugar by inducing resistance to the activity of excessive quantities of insulin. Secondly, it

appears to us that use of steroids may prevent the mysterious "idiopathic hyperthermia" which caused five of the eighteen postoperative deaths in the series of cases culled from the literature by Howard.[7] All hyperpyrexic deaths occurred within four days of removal of a benign adenoma. In the past two years a solitary insuloma has been excised from four patients at University Hospital. Three of these patients had received 120 units of aqueous ACTH intramuscularly daily pre- and postoperatively and all recovered uneventfully from their surgery. The fourth patient received no ACTH or steroids. Thirty-six hours postoperatively his temperature rose suddenly to 107°F. and he expired within two hours. No explanation for the hyperthermic crisis was disclosed at autopsy. This experience was probably nothing more than a coincidence but it deserves serious thought. A number of theoretic possibilities exist to explain this phenomenon.

At laparotomy, the surgeon can now be guided by the statistical data regarding the location, size and detectability of benign adenomas. Of 274 functioning adenomas[7] the tumors were almost equally distributed in the head, body and tail. Ectopic tumors are rare and are usually contiguous with the pancreas (in the duodenal wall, in the splenic hilum or posterior to the pancreas). Functioning benign tumors are usually of small size, the diameter in 75 per cent of them ranging from 1 to 3 cm. and rarely exceeding 5 cm.[5,6] Tumors of the body and tail tend to lie near the anterior surface of the pancreas and to be visible or palpable; whereas those in the head are usually buried deeply within the parenchyma.[116,117] Adenomas are usually of firmer consistency than normal pancreatic tissue, and are therefore palpable when not visible as purplish nodules.[20] However, de Peyster[118] reports three cases of soft, non-palpable tumors which were detectable only after the excised gland had been resected.

When no tumor is discovered in the body or tail of the pancreas, the surgeon must proceed with a meticulous exploration of the head after mobilizing the second portion of the duodenum before he considers subtotal resection. This important point has been emphasized repeatedly.[20,117,119,120] The literature contains many reports of tumors later removed from the head of the pancreas when symptoms persisted following subtotal resection. Statistically, a "blind" subtotal resection should remove 62 per cent of the tumors. However, the postoperative mortality after excision of a portion of the pancreas. (14 per cent) was significantly greater than that following simple removal of an adenoma (9 per cent).[7] The surgeon's task is complicated further by the fact that multiple adenomas occur in 10 to 15 per cent of the cases.[7]

An interesting observation, which eventually may be helpful to the surgeon, was made by McMillan.[121] While operating upon a patient with organic hyperinsulinism, he obtained blood samples for glucose determination immediately before and thirty minutes following removal of suspiciously abnormal tissue; and awaited the results before closing the abdominal wound. The blood sugar rose from 131 to 258 mg. per cent. This made him reasonably certain that he had removed the adenoma. During a previous operation on the same patient there had been no such rise in blood sugar when lymphoid tissue had been removed instead of the insuloma. One might argue that the frozen section gives the same information, but in the case of multiple tumors it would not. De Peyster[118] and Dickie[122] report similar hyperglycemia responses within ninety minutes following removal of a tumor. More observations of this kind are needed.

If the patient refuses surgery or if great obesity constitutes a prohibitive surgical risk, alternative management should be attempted on a temporary basis. Hydrocortisone can then be employed in sufficient dosage to maintain normal fasting blood sugar levels. Large doses of steroid will be required, and should be used only as a temporary expedient while the patient is being convinced of the need for surgery or is being dieted down to a weight compatible with adequate pancreatic exploration. Large doses of steroids should, of course, be used to prevent frequent hypoglycemic attacks produced by metastatic islet cell carcinomas. Alloxan has been unsuccessful in controlling organic hyperinsulinism caused by either benign[113] or malignant islet cell tumors.[123,124] Administration of an extract of the adenohypophysis, which produced permanent diabetes with islet cell degeneration in dogs, intensified the hypoglycemia in two patients with organic hyperinsulinism.[125]

Hepatogenic Hypoglycemia

Proper perspective is required in assessing the role of liver disease as a cause of hypoglycemic episodes. In relation to the large total number of

patients with severe hepatocellular disease, those who exhibit symptomatic hypoglycemia are few. Nevertheless, the absolute number is probably considerably larger than is generally appreciated. This view is strengthened by reports that previously unsuspected hypoglycemia of hepatic etiology was the principal cause of symptoms in several situations in which attention had been focused upon more obvious clinical manifestations.[32,34]

Hepatogenic hypoglycemia was first recognized in patients with advanced and irreversible lesions of toxic or neoplastic nature.[22,23,25–27] Similar cases are still described.[28] Hypoglycemia as the dominant cause of symptoms in some patients with reversible hepatocellular lesions has been established.[21,24] Recent reports that profound hypoglycemia can precipitate mental torpor masquerading as "hepatic coma" in Laennec's cirrhosis[32] or as "cerebral anoxia" in the chronic passive congestion of heart failure,[34] lend credence to the view that many cases of hepatogenic hypoglycemia are not recognized.

The clinical and laboratory criteria required for a diagnosis of hepatogenic hypoglycemia have been outlined in Table II. The characteristic response to ingested glucose is demonstrated in Figure 1. Impairment of hepatic glycogenesis as well as of hepatic glycogenolysis account for the disturbance of carbohydrate metabolism in the advanced case.[21] Fortunately, these functions are maintained in the majority of patients with hepatic disease for a longer time than are many of the other functions for which tests are available. From a diagnostic point of view three elements stand out as the critical ones: (1) inability to tolerate fasting or low carbohydrate feeding without severe hypoglycemia; (2) evidence, from multiple tests, of diffuse hepatocellular disease; and (3) a plateau glucose tolerance curve not brought down by preparation with diets very high in carbohydrate. In rare instances in which organic hyperinsulinism occurs in a patient with severe hepatic disease, the differential diagnosis will be difficult indeed, if not impossible.

In severe acute hepatic lesions of infectious or toxic etiology when hypoglycemia is present, recovery of the blood sugar regulatory functions of the liver may be expected to parallel restoration of other hepatic functions.[21,32,34]

Illustrative Case. In September, 1951, I. R., a thirty-two year old housewife, noted the onset of weakness, ease of fatigue, and stabbing postprandial upper abdominal pain. Anorexia and chronic nausea developed. Jaundice appeared five weeks after the onset of symptoms, and was associated with pruritus, dark urine and clay-colored stools. X-ray examination elsewhere led to a diagnosis of obstructive gallbladder disease. At laparotomy the gallbladder was reportedly enlarged and thickened; and although it contained no stones or gravel, cholecystectomy was performed. Postoperatively the jaundice, pruritus and anorexia abated for several weeks, but all symptoms recurred with progressive intensity. Episodes of colicky right upper quadrant pain occurred, radiating to the right scapular area. Total loss of weight from the onset of illness was 20 pounds. On March 4, 1953, the patient was admitted to University Hospital.

Except for intense jaundice she appeared neither acutely nor chronically ill. The oral temperature was 98.6°F., pulse 92, blood pressure 110/70, height 67 inches and weight 168 pounds. A soft, sharp, tender liver edge was palpable 6 cm. below the right costal margin on deep inspiration. The spleen was not palpable.

The only abnormalities in routine blood and urine studies were a positive Harrison spot test for bilirubin and a moderate increase in urine urobilinogen. The stool was light brown in color. Liver function tests revealed the following values: total serum bilirubin, 37.4 mg. per cent, with one-minute direct bilirubin, 14.1 mg. per cent, thirty-minute direct bilirubin, 23.2 mg. per cent and indirect bilirubin, 14.2 mg. per cent; gamma globulin, 33.0 units; thymol turbidity, 15.7 units; cephalin cholesterol flocculation, 4-plus after both twenty-four and forty-eight hours; total serum cholesterol, 42 mg. per cent, 25 per cent of which was esterified; prothrombin concentration, 27 per cent; alkaline phosphatase, 11.8 King-Armstrong units; total serum protein, 6.1 gm. per cent, with albumin, 2.9 gm. per cent and globulin, 3.2 gm. per cent.

Strict bed rest, a diet high in protein and carbohydrate, and vitamin supplements were prescribed. On the seventh hospital day the patient complained of nausea in the morning. Consumption of food on this day was negligible. At 3:45 P.M. she noted the abrupt onset of weakness, faintness, sweating and trembling. Blood pressure was 136/66, pulse 100. A blood sample for glucose determination was obtained and 50 per cent glucose was given intravenously. The symptoms disappeared at once. The blood sugar level at the time of

symptoms was 23 mg. per cent. The following morning the fasting blood glucose was 52 mg. per cent. Two months after the hypoglycemic episode the fasting blood sugar was 83 mg. per cent. Convalescence was slow and without relapse, but two full years passed before all tests of liver cell function had returned to normal. The etiology of the profound hepatocellular damage was presumed to be viral. It is interesting to speculate, in passing, that temporary relief of the jaundice following cholecystectomy was associated with a "stress-induced" activation of the pituitary-adrenal system.

Management. In most cases dietary management is all that can be offered. The high carbohydrate, high protein diet generally prescribed in hepatocellular disease, but with the addition of a bedtime meal to shorten the longest fasting period of the day, eliminates the hypoglycemic attacks which usually occur before breakfast. In the rare instance in which liver cell damage is secondary to an active ascending biliary tract infection, cholecystectomy may result in sufficient return of hepatic function to ensure normal carbohydrate metabolism.[21]

Idiopathic Spontaneous Hypoglycemia of Infancy and Childhood

Perhaps the most interesting recent development in this field is McQuarrie's careful delineation of the most common form of spontaneous hypoglycemia encountered in early childhood.[51] Not only does his analysis explain the mystery of the many older reports of infantile hypoglycemic convulsions in the absence of a detectable organic lesion,[7,114,115] but it also affords a simple and effective substitute for subtotal pancreatectomy.

The virtually complete absence of alpha cells in the islets of two young siblings suffering from hypoglycemic attacks led McQuarrie originally to postulate a congenital deficiency of the hyperglycemic-glycogenolytic "alpha cell factor" (glucagon) as the etiologic lesion.[126] The validity of this concept has been shaken, however, by failure to find a paucity of alpha cells in pancreatic biopsies obtained from subsequent patients. Properly, McQuarrie is unwilling now to localize the primary lesion to the pancreatic islets, because of the possibility that a pituitary insulotropic principle[125] or a hypothalamic abnormality[127] may be the stimulus causing hyperinsulinism. Examination of his data leaves little doubt that this is a type of hyperinsulinism (relative or absolute) indistinguishable from that caused by islet cell tumors in adults. The hypoglycemia is of the fasting variety, but in addition the glucose tolerance test demonstates the stimulative component, namely, a sharp secondary postprandial hypoglycemia. Although the histologic appearance of the islets is usually normal, the data are highly suggestive that excessive insulin activity is present both in the fasting and in the postprandial state. A high familial incidence (siblings and cousins) is reported.

It is noteworthy that the onset of hypoglycemic manifestations varies from the first day of life to the age of five years; and that no clinical or laboratory evidence of endocrine, hepatic or gastrointestinal disease is present. Twenty-one of McQuarrie's twenty-five patients were symptomatic before the age of two years. The clinical picture is not duplicated by other types of infantile hypoglycemia. Newborn babies demonstrate moderate fasting hypoglycemia for about two weeks, but this is asymptomatic and not progressive.[51,128–130] Occasionally a baby born of a diabetic mother develops symptoms of fasting hypoglycemia,[14,51,131] but again these are transient. During infancy some children manifest a postprandial type of hypoglycemia due to overstimulation of normal islet cells by excessive ingestion of sugar (Staub-Traugott phenomenon).[51] This is a stimulative type of hypoglycemia, the fasting blood sugar remaining normal and symptoms subsiding upon relative restriction of carbohydrate.

Idiopathic infantile hypoglycemia is not a permanent condition, but one which subsides spontaneously as the child grows older. McQuarrie emphasizes the tragedy of unrecognized cases, in which permanent brain damage remains after the hypoglycemia (which could have been controlled) has disappeared.

Management. McQuarrie believes that corticotropin is the treatment of choice; that cortisone or hydrocortisone are less effective.[51] Twenty-five mg. of ACTH gel daily may be required initially, but with time as little as 2 mg. every other day has been sufficient to prevent hypoglycemia. In four such patients, discontinuation of ACTH therapy has been possible after several months without return of hypoglycemia. In view of the success of this simple regimen in a self-correcting condition, exploration of the pancreas for an islet cell tumor (very rare in this age group) should be deferred and the possibility

re-evaluated at a later date pending future developments.

Illustrative Case. Patient L. T., a Negro girl five months of age, was admitted to the Pediatrics Division of University Hospital on December 13, 1948. She had been a normal, full term baby with no evidence of birth injury. At the age of two months she began to have episodes of jerking of the head, rolling of the eyes and "blankness." Attacks occurred every two or three days, and were not associated with fever. They lasted only a few minutes and always subsided spontaneously. Episodes were never noted within an hour after feedings. The drowsy state which followed an attack disappeared more quickly if the patient was given milk or fruit juice. Before the morning feeding on the day of admission she had had an unusually severe attack. When an electroencephalogram was said to be compatible with a grand mal convulsive disorder, the patient was discharged on a regimen of phenobarbital and dilantin.

From December, 1948, until August, 1953, she was seen periodically in the Pediatrics Clinic. During this time frequent generalized seizures had occurred despite anticonvulsant therapy, and progressive mental deterioration was observed. She was readmitted on August 24, 1953, at the age of six years, following a three-day episode of diarrhea and anorexia. She had had five severe convulsions on the day of admission.

The patient was found to be comatose, with a rectal temperature of 100°F. Frequent twitching of the extremities and occasional generalized seizures occurred intermittently. A spinal fluid sugar of 4 mg. per cent and a blood sugar of 14 mg. per cent were found. After three days of an infusion of glucose, she regained consciousness. A permanent left convergent strabismus was then noted.

Fasting blood sugars ranged from 24 to 39 mg. per cent. Oral glucose tolerance test showed: fasting, 39 mg. per cent; one-half hour, 157 mg. per cent; one hour, 137 mg. per cent; two hours, 136 mg. per cent; three hours, 92 mg. per cent; four hours, 50 mg. per cent; five hours, 29 mg. per cent. The hyperglycemic response to epinephrine and a battery of liver function tests were entirely normal.

On October 16, 1953, careful exploration of the entire pancreas disclosed no adenoma. The tail and body of the pancreas, comprising approximately 70 per cent of the gland, were resected. Histologic examination revealed some increase in the number and size of the islets of Langerhans. Staining of the islets by the Gomori technic demonstrated a normal ratio of alpha and beta cells.

Hypoglycemia has not recurred. The morning after surgery the fasting blood sugar was 192 mg. per cent, two weeks later it was 126 mg. per cent, and fifteen months later it was 90 mg. per cent. However, mental retardation persists.

REMARKS UPON SOME OF THE RARER CAUSES OF SPONTANEOUS HYPOGLYCEMIA

Finally, a few additional comments are indicated upon some of the rarer causes of spontaneous hypoglycemia, which we have included in bringing up to date our older etiologic classification.[15]

1. Hypoglycemia associated with primary hypothyroidism: Two more cases[51,52] of severe hypoglycemia responding to administration of thyroid substance have been reported. We continue to believe that hypothyroidism, *per se*, is not a cause of symptomatic spontaneous hypoglycemia. Lack of function of the adenohypophysis and of the adrenals as the result of long-continued primary hypothyroidism (myxedema of the pituitary, adrenal, etc.) is the more likely explanation. Administration of thyroid repairs their responsiveness. While this would appear to be splitting hairs, it is stated in an effort to avoid cloudy concepts.

2. Hypoglycemia associated with fibromas and sarcomas: There have now been reported twelve cases of large sarcomatous or fibromatous tumors associated with a severe, fasting type of hypoglycemia.[36,61,132] Eight of these tumors were excised with prompt disappearance of hypoglycemia. Recurrence of the tumor has occurred in five, with return of hypoglycemic attacks. Two of the tumors were assayed for insulin and none was found to be present.[36,136] Nevertheless, it has been postulated[61] that these tumors secrete insulin or something akin to it. In view of the great size of these neoplasms it would seem more likely that they are consuming selectively very large amounts of glucose.

3. Hypoglycemia associated with congenital adrenal hyperplasia: In 1951, White and Sutton[60] reported the occurrence of episodes of severe hypoglycemia in a four year old female with congenital adrenal virilism. These attacks occurred

always under conditions of stress and were not associated with excessive loss of sodium. Subsequently, Wilkins[137] also observed stress-induced hypoglycemia, without abnormality of electrolyte metabolism, in a twenty-six month old male with macrogenitosomia precox. We[138] have seen the same phenomenon in a two year old girl with congenital adrenal hyperplasia. On two occasions profound hypoglycemia occurred in the course of acute infection. In this patient, however, temporary severe depletion of sodium accompanied the hypoglycemic crises. We have also observed abnormally low levels of blood sugar in two infants with this same disease, in whom the primary problem was severe loss of sodium.

It is reasonably clear now that a fundamental disturbance in congenital adrenal hyperplasia is an inability of the adrenal cortex to produce normal amounts of hydrocortisone.[139] Deficiency of the finished product in the body constitutes an abnormally and continuously great stimulus for increased production of ACTH. The adrenals become hyperplastic and produce a large amount of steroidal material other than hydrocortisone, much of which appears in the urine as 17-ketosteroids. Thus it is not surprising that under conditions of stress, ordinarily accompanied by a sharp increase in the level of hydrocortisone in the blood,[140] some patients with this disturbance manifest symptomatic hypoglycemia. Ely et al.[141] have reported that both the fasting blood sugar and the concentration of 17-hydroxycorticoids of plasma are significantly lower in these patients than in normal children of the same age.

At present hydrocortisone is the treatment of choice in the management of congenital adrenal hyperplasia. ACTH is contraindicated. It intensifies the abnormalities which characterize the condition.

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Clinico-pathologic Conference

Jaundice and Ascites with Recovery for Two Years; Recurrence of Jaundice, with Abdominal Pain, Hematemesis and Azotemia

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D., of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Interna Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A FIFTY-NINE year old white male bus driver (No. 241074) entered the Barnes Hospital for the first time on July 31, 1954, with a chief complaint of jaundice of three weeks' duration.

For the past five or six years the patient had noted occasional mild epigastric discomfort associated with bloating following meals. About four or five years prior to admission he suffered the onset of intermittent right upper quadrant throbbing pain, particularly postprandially, which was aggravated by eating pork or cabbage. Approximately two years prior to admission the patient noted an increase in the severity of this discomfort, at which time he had the onset of non-pruritic jaundice and an increase in the girth of his abdomen, associated with anorexia, occasional vomiting and ankle edema. He was hospitalized elsewhere for fifty-five days, during which time five paracenteses were performed, with removal of 4 to 5 quarts of ascitic fluid each time. His jaundice, ankle edema and ascites gradually disappeared. His appetite improved and he returned to his job. He felt well until approximately five weeks prior to this admission; while cutting weeds he became very fatigued and noted again the onset of right upper quadrant pain, this time radiating around the right flank and across the abdomen. This pain subsequently occurred following meals. He had a gradual decrease in his appetite with some increase in abdominal bloating and flatulance. He also began to have occasional vomiting, particularly in the morning on arising. Three weeks prior to admission the patient noted the onset of jaundice associated with dark urine, light stools, a bad taste in his mouth, increasing weakness, an increase in the girth of his abdomen and more frequent vomiting during the day.

Prior to two years before admission the patient ingested alcohol only rarely on social occasions. He denied drinking alcohol at all for two years prior to admission. There had been no known exposure to hepatotoxic agents. He had lost a total of 10 pounds during the previous six weeks. His diet was not described in detail but was stated to have been "adequate."

Physical examination on admission revealed the patient's temperature to be 37°C.; pulse, 80; respirations, 16; and blood pressure, 130/80. The patient was a well developed man appearing chronically ill and mildly jaundiced. There were several arteriolar spiders on the upper trunk, but no palmar erythema. There were a few shotty inguinal lymph nodes. The sclerae were icteric, the pupils reacted to light and accommodation, and extraocular movements were intact. The eyegrounds revealed slight increase in the light reflex with some questionable temporal pallor. Examination of the ears, nose, mouth and throat was not remarkable. There was moderate increase in the anteroposterior diameter of the thorax. Examination of the lungs revealed hyperresonance on percussion with a few coarse rhonchi over the left chest anteriorly on auscultation; no rales were heard. The left

border of cardiac dullness was encountered 8 cm. from the mid-sternal line in the fifth intercostal space. The heart sounds were of good quality, no murmurs were heard, and there was a normal sinus rhythm. The abdomen was slightly protuberant; there was some bulging of the flanks and shifting dullness, but no fluid wave. There was poorly defined tenderness in the right upper quadrant. Liver, kidneys and spleen were not palpable. There was a readily reducible, left, indirect, inguinal hernia. There was a trace of pitting edema of both ankles. Rectal examination revealed a firm prostate which was two or three times normal size, was not tender and contained no nodules. No hemorrhoids were noted. The remainder of the physical examination was not remarkable.

The past history disclosed that in 1923 the patient had had pneumonia. Since then he had noted asthmatic attacks usually in the fall and the winter. One brother had asthma.

Laboratory data on admission were as follows: hemoglobin, 12.1 gm. per cent; white blood count, 6,800 per cu. mm.; differential: basophils, 1 per cent; segmented forms, 69 per cent; lymphocytes, 30 per cent. Urinalysis: pH, 5.5; protein, negative; sugar, negative; microscopic examination: amorphous urates, 4 plus; bacteria, 2 plus. Stool: guaiac-negative. Serologic test for syphilis: negative. Blood chemical determinations were as follows: non-protein nitrogen, 15 mg. per cent; fasting blood sugar, 55 mg. per cent; sodium, 137.5 mEq./L.; potassium, 4.4 mEq./L.; chloride, 100 mEq./L.; carbon dioxide combining power, 28.2 mM/L.; cephalin cholesterol flocculation, 4 plus; thymol turbidity, 38.6 units; total bilirubin, 6 mg. per cent; one minute fraction, 2.4 mg. per cent; albumin, 3 gm. per cent; globulin, 6.2 gm. per cent; cholesterol, 167 mg. per cent; amylase, 107 Somogyi units; prothrombin concentration, 22 per cent of normal; alkaline phosphatase, 5.8 Bodansky units; bromsulfalein retention, 64.5 per cent at the end of forty-five minutes. Electrocardiogram: within normal limits. Roentgenogram of the chest: minimal infiltrate in the left upper lobe which was interpreted as representing a questionable fibrotic tuberculous lesion; hypertrophic osteoarthritis of the dorsal spine; elongation of the aorta. Complete gastrointestinal series: within normal limits. Gallbladder series: non-visualization of the gallbladder. Sputum culture: a heavy growth of neisseria and a moderate growth of beta-hemolytic streptococci.

The patient's hospital course was uneventful until the sixth hospital day, at which time he began to complain of severe aching pain in the right flank, radiating inferiorly to the pubis and questionably to the right side of the scrotum. He denied dysuria, urinary frequency or urgency. At that time he manifested extreme tenderness in the right costovertebral angle with radiation of the tenderness anteriorly around the right flank, and his temperature spiked to 38.5°C. Urinalysis was as follows: pH, 4.5; protein, negative; sugar, negative; microscopic examination: mucus, 3 plus; 30 to 35 white blood cells per high power field, moderate number of epithelial cells, rare red blood cells, rare white blood cell casts. A urine culture grew a few colonies of Staphylococcus albus and Staphylococcus aureus, and a moderate growth of beta-hemolytic streptococci. He was given tetracycline, 250 mg. orally every six hours. However, this dose was changed to 200 mg. intramuscularly four times a day because of vomiting which began after three oral doses of the medication; the intramuscular drug was continued throughout the remainder of his hospital stay. By the following day he had been given three doses of atropine, 0.4 mg. each dose. His discomfort had decreased but was still present. His abdomen was distended and there was tenderness in the epigastrium and the right upper quadrant. A rectal examination at that time was not remarkable; there were no feces in the rectal ampulla. The white blood count on the seventh hospital day was 14,750, the differential count comprising myelocytes, 6 per cent; metamyelocytes, 19 per cent; band forms, 50 per cent; segmented forms, 20 per cent; lymphocytes, 4 per cent; monocytes, 1 per cent. Slight hypochromasia of the red blood cells with adequate platelets was noted and two atypical lymphocytes were seen. Roentgenograms of the chest and abdomen: discoid atelectasis at the base of the right lung, and ascites; because of the absence of gas in the rectum and large bowel it was believed that a very early small bowel obstruction could not be excluded on the basis of this roentgen examination. The patient continued to vomit and was maintained on parenteral fluids. On the morning of the eighth hospital day retrograde pyelograms were taken; these were within normal limits. The patient's emesis became coffee-ground in quality. On the afternoon following the retro-

grade pylography the patient's blood pressure was found to be 74/40 with a pulse rate of 120. An electrocardiogram showed no change. He was given vasoxyl,® 20 mg. intramuscularly, with subsequent rise in blood pressure to approximately 100/60. The patient was then placed on an intravenous drip of nor-epinephrine in the amount of 4 mg./L.

The patient's condition rapidly deteriorated. The serum non-protein nitrogen increased progressively to a high of 174 mg. per cent on the fourteenth hospital day at which time the serum potassium was at its height, 7.4 mEq./L. Despite electrolyte and fluid replacement his serum sodium remained low, in the range of 125.8 mEq./L. to 132.1 mEq./L. He was maintained on almost constant gastric suction. Intravenous nor-epinephrine was continued until the eleventh hospital day, but it had to be reinstituted on the thirteenth day because of a gradual fall in the blood pressure in the previous twelve hours to a low of 75/35. The patient's jaundice became more intense His urine output decreased from 500 ml. on the seventh hospital day to only 100 ml. on the thirteenth hospital day. He was given large doses of parenteral vitamins in addition to sodium glutamate, 23 gm. per day, for three days beginning on the eleventh hospital day. The prothrombin concentration fell to 15 per cent of normal despite administration of parenteral vitamin K, 20 mg. each day, beginning on the fifth hospital day. Fresh ecchymoses at pressure sites were noted on the eleventh hospital day. The patient became intermittently irrational, and died quietly on August 13, 1954.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: I would like to start the conference by having Dr. Elliott show us the x-ray films.

DR. GLADDEN ELLIOTT: The first examination was of the chest, and one of the few positive findings suggested was a questionable infiltrate of the second anterior interspace representing minimal fibrotic pulmonary tuberculosis. The remainder of the chest was normal. A gastrointestinal series was negative, and a retrograde pyelogram on August 7th showed normal collecting systems bilaterally. At that time the patient had a rather marked degree of ascites.

DR. REINHARD: This patient, a fifty-nine year old bus driver, had only one admission to the Barnes Hospital, and he died two weeks after this one admission. He was not an alcoholic; his diet is stated to have been adequate, although a detailed description was not available. About six years prior to this admission he had noted occasional mild epigastric discomfort associated with postprandial bloating. Two years prior to admission abdominal pain developed. The intern's note stated that this was a right upper quadrant throbbing pain; the assistant resident's note described this as a right lower quadrant pain which was constant, dull and aching in character. Two years prior to admission an increase in severity of this pain developed, associated with non-pruritic jaundice, an increase in the girth of his abdomen, and anorexia, occasional vomiting and ankle edema. He was hospitalized for two months, during which period five paracenteses were performed, each one yielding from 4 to 5 quarts of ascitic fluid. At the end of this two months of hospitalization his jaundice had subsided, his ankle edema had cleared up, his ascites had disappeared and he was asymptomatic. As far as we know he was entirely well so far as any residual evidence or clinical symptoms were concerned. He returned to work following this and remained well for a period of two years. In June, 1954, after two years of freedom from any symptoms, symptoms referable to his terminal illness began to develop. This was five weeks prior to his admission to the Barnes Hospital. Dr. Shank, this patient was not seen during the two-year interval when he was asymptomatic, so we have inadequate data about him during this period. If this patient had come to see you in June, before any of his terminal symptoms developed, saying he had felt perfectly well for two years but giving you this interesting history, how would you have interpreted this past story?

DR. ROBERT E. SHANK: Under the conditions that you have outlined I would have wanted to review the history very carefully and obtain a little more data than our protocol contains. With the information that we have available to us in this synopsis of his history, it seems to me that there are several possibilities, each difficult to assess. One of these possibilities is that cirrhosis had developed at the time of his final illness, either on the basis of a nutritional deficiency or as the result of previous hepatitis. For that reason I would have liked to know what this man's dietary intake was five or six years ago rather than in more recent months.

DR. REINHARD: I am sure we both agree that

the dietary history available was totally inadequate.

DR. SHANK: Another possibility is that this man may have previously had an acute illness involving his liver, the most likely type being viral hepatitis. If that were true, hepatitis would have been occurring in a man of relatively advanced age; it is a much more common disease in earlier life. On the other hand, it does occur in older age groups and is frequently associated with a prognosis that has to be more guarded. In other words, the man would have been more likely to have had a severe course, because he was older than fifty at the time of onset. If he had had acute hepatitis at that time, we would have to postulate that the course of this acute illness was not the usual one, going on to complete cure, but that he had had a period of prolonged convalescence with the persistence of some symptoms and the evolution of a chronic lesion following his acute hepatitis. Regardless of the initial type of liver disease he could have had remissions and exacerbations. In the course of his illness this man undoubtedly had a rather long period of remission of his disease, but this does not help us decide what was the initial event in his illness. Something had happened at the time of his final illness to cause an exacerbation of his disease.

DR. REINHARD: Would you like to favor one or the other of these types of chronic liver disease?

DR. SHANK: If you will put it in the category of a guess, I will make one. When first seen here this man did not have a palpable liver or spleen. If his primary disease had been portal cirrhosis induced by dietary deficiency, his liver probably would have been palpable and with this course his spleen also might have been palpable. However, there are instances when significant hepatomegaly is absent in portal cirrhosis. If, on the other hand, he had had postnecrotic cirrhosis, it would have been more likely that his liver would not have been palpable when he was seen here. On that basis, then, we could lean a little more to the initial diagnosis of acute hepatitis progressing to postnecrotic cirrhosis. I would like to stress that the initial disease would not necessarily have had to be viral hepatitis. It could have been acute liver damage of some other type, for example, that due to hepatotoxins.

DR. REINHARD: Dr. Karl, will you comment on this fact: this patient has ascites requiring five paracenteses in a period of two months; the ascites then cleared up and he required no further taps for a period of two years. Do you think this constitutes very convincing evidence against either type of cirrhosis?

DR. MICHAEL M KARL: I think it would not provide evidence against either type. One must remember that in the production of ascites in liver disease there are many factors involved other than portal hypertension. These include an excess of antidiuretic substance, sodium retention presumably on a hormonal basis, changes in the levels of the serum protein, and changes in the permeability of the capillaries. All of these are conditions in which reversible factors are operating. These might well change in the course of either of these two conditions under consideration.

DR. REINHARD: Have you seen this?

DR. KARL: Yes, I have.

DR. REINHARD: Dr. Shank, how often does a patient have ascites which then clears up? Is this very common?

DR SHANK: This is not at all uncommon in carefully managed patients.

DR. REINHARD: Many different varieties of chronic hepatitis have been described and many names have been applied to differentiate the variations in the clinical picture following acute hepatitis. One type is the benign condition called in the literature posthepatitis bilirubinemia which may be persistent or intermittent. A second category is acute infectious hepatitis with "apparent" recovery, "apparent" because these patients end up with advanced cirrhosis. Therefore it would appear that during this period of freedom from any symptoms or manifestation of disease, there must have been some latent process which caused progressive damage to the liver. The third category of chronic active infectious hepatitis may be of various types. It may be characterized by continuous or recurrent jaundice, or by persistent symptoms and laboratory evidence of hepatic dysfunction. Jaundice may be absent but the patient may have various vague symptoms of anorexia, bloating and nervousness without any physical signs or laboratory evidence of hepatic dysfunction. These patients are often diagnosed as having psychoneurosis. Indeed, many of them may be patients with psychoneurosis who had active hepatitis in the past and whose exacerbation of psychoneurosis dates from the acute attack of hepatitis. The fourth category has been called chronic inactive infectious hepatitis. This may again be with or without

jaundice. This is a rather vague category, hard to distinguish from the other cases of chronic active infectious hepatitis. In many instances there may have been very little to indicate actual activity of the disease process. Dr. Mendeloff, would you care to tell me first whether you think this patient had primarily hepatitis or nutritional liver disease?

DR. ALBERT I. MENDELOFF: At the time this patient entered this hospital he presented the signs of chronic liver disease, most probably cirrhosis. When one tries to differentiate among the specific types of cirrhosis in a case in which the history is as unrevealing as this one was, one has to use a number of other findings, either laboratory or physical, which statistically are more often associated with one type than another. As Dr. Shank pointed out, in this case the liver was not palpable, nor was the spleen. The patient had a few spider angiomas, was moderately jaundiced, and had some fluid in his abdomen. The fact that his liver and spleen were not palpable is, statistically, most often associated with the postnecrotic form of chronic liver disease, whether postnecrotic scarring alone or whether postnecrotic cirrhosis, the more active form. The laboratory data tend to confirm this again from a statistical point of view. They reveal several features which are more often associated with the postnecrotic type of cirrhosis than with a diffuse cirrhosis. First the serum globulin was 6.2 gm. per cent. This is an extremely high value for either disease, but values over 5 are far more common in the form of disease which we call postnecrotic cirrhosis. Second, the normal alkaline phosphatase is in keeping with postnecrotic cirrhosis. The very high thymol turbidity and a very high cephalin cholesterol flocculation are not very helpful in differentiating the two types. I do not believe they help us one way or the other. I would say that both clinical findings and the laboratory data tend to favor postnecrotic cirrhosis in this case although, as everyone knows, none of these points is conclusive.

DR. REINHARD: After this asymptomatic two-year period, on June 12, 1954 (five weeks before death), the patient became very fatigued and noted the occurrence of right upper quadrant pain while cutting weeds on a friend's farm. This pain radiated around the right flank and down to the lower abdomen. Subsequent recurrences of this pain occurred and seemed to come on more often after meals. A progressive increase in anorexia, bloating and edema developed. He began to have occasional vomiting spells, particularly on arising in the morning. Shortly thereafter he noted the onset of jaundice. This was again without pruritus. He had dark urine and a bad taste in his mouth, weakness became steadily more marked, and there was an increase in the size of his abdomen. More frequent vomiting occurred during the day. The day before admission his vomitus contained a small amount of blood for the first time. On the day of admission his stool was the color of light cream for the first time. During the six weeks prior to his admission to the hospital he lost 10 pounds. The patient had had no chills, he did not think he had had any fever, and upon admission to the hospital he did not have any fever. On physical examination his temperature, pulse and respiration were normal. The patient was mildly jaundiced. There were several arterial spiders on the upper trunk. He had no palmar erythema. The scleras were icteric. The abdomen was slightly protuberant. There was some bulge in the flanks and shifting dullness, but no fluid wave was noted. The liver, spleen and kidneys were reported by several investigators to be not palpable. There was a trace of pitting edema of both ankles. He had no hemorrhoids. Dr. Kenamore, this was your patient. When you saw him at this time, what diagnoses did you consider as the cause of the rather sudden onset of acute symptoms?

DR. BRUCE KENAMORE: When I first saw this man, about two or three days before he entered the hospital, I thought he either had had an exacerbation of cirrhosis which resulted from acute hepatitis with prolonged course occurring two years before, or, lacking any laboratory data at the time I first saw him, that he had obstructive jaundice. He was obviously ill and in need of hospitalization and nothing more was done in the way of history or physical examination until he was admitted to the hospital. Those were the two considerations in my mind at the time of admission.

DR. REINHARD: On admission he had mild anemia, hypoglycemia, a low serum cholesterol, a prolonged prothrombin time, markedly elevated cephalin cholesterol flocculation and thymol turbidity, profound bromsulfalein retention, and a serum bilirubin markedly elevated in both direct and indirect fractions. Dr. Recant, do these findings help us any in determining what the nature of this insult to the liver was?

DR. LILLIAN RECANT: The only conclusion one could reach from these findings is to say that there was no evidence of any obstructive phenomenon, and that there had been severe hepatocellular damage.

DR. REINHARD: On the sixth hospital day the patient began to have severe aching pain in his right flank. This radiated down to the genitalia. There was extreme costovertebral angle tenderness. He had fever at this time, 20 to 30 white cells in the urine, and very few red cells. Urine culture grew out beta-hemolytic streptococcus and only a few straphylococci. The day after these events he had a white blood cell count of 14,700, with a very striking shift to the left. Dr. Harford, was this a separate urinary tract infection, or was this a renal disease due to or associated with his liver disease?

DR. CARL G. HARFORD: This probably was a urinary tract infection.

DR. REINHARD: What organism would you have suspected here?

DR. HARFORD: The laboratory isolated a streptococcus that gave beta-hemolysis. One would not expect, it seems to me, to have urinary infection due to group A streptococci; however, there are some enterococci encountered in the urine which would also give beta-hemolysis, so I imagine they were the organisms isolated.

DR. REINHARD: I have never seen so marked a shift to the left with a total white cell count of only 14,000 in the presence of a hemolytic streptococcal infection. I am a little disturbed by this. I have seen this more often in association with profound necrotic disease—advanced carcinoma with profound necrosis or, perhaps, with profound liver necrosis. Does this white blood cell count disturb you?

DR. HARFORD: Yes, it does disturb me. All the rest of the circumstances are such as to indicate that any urinary tract infection which was present would probably have been fairly promptly controlled by the medication given.

DR. REINHARD: This patient began vomiting continuously thereafter. The day after the onset of these acute symptoms his abdomen became distended. A small bowel obstruction was suspected by at least one observer. On the eighth hospital day, two days after the onset of acute symptoms of fever and the like, the patient vomited coffee ground material. That same day, in spite of the fact that his non-protein nitrogen concentration was 62 mg. per cent, bilateral retrograde pyelography was performed. The following day the patient went into shock which was treated with vasoxyl and norepinephrine. Jaundice became progressively more intense and his urinary output steadily decreased, finally reaching anuric levels. During the last two days of his illness his blood pressure fluctuated between 80 and 105 systolic and 50 and 55 diastolic. On the day of death the sodium was low and the potassium was high. Dr. Sherry, what kind of kidney disease do you think this patient had, and would you comment on these terminal events?

DR. SOL SHERRY: About five, six or seven days after some patients have had a very severe acute hepatic insult, they will suddenly go into acute renal insufficiency. I think that the pathologist will show us evidence of acute tubular necrosis; I believe this case was an example of the hepatorenal syndrome. The terminal events might almost suggest that there had been some adrenal insufficiency of a physiologic nature as well.

DR. REINHARD: Do you think that taking the retrograde pyelograms when the patient was azotemic was ill-advised, or did this have nothing to do with the terminal events?

DR. SHERRY: I doubt that it had anything to do with the terminal events.

DR. REINHARD: Dr. Harrington, would you discuss this patient's terminal bleeding difficulties? Just before he died bleeding from his gastrointestinal tract developed which may or may not have been due to a localized gastrointestinal lesion. He also certainly had a blood dyscrasia of some sort because he had a great many spontaneous ecchymoses occurring during the last few days of life. We all know the patient had a profoundly low prothrombin concentration in his blood. Are there any other mechanisms for the bleeding that we would have had to consider?

DR. WILLIAM HARRINGTON: Yes, in the terminal stages of liver disease many abnormalities may be noted. Deficiency of prothrombin, accelerator globulin and convertin all contribute to prolongation of the one-stage determination of prothrombin time. In addition, hypofibrinogenemia can occur, although it is an unusual cause for generalized bleeding in cirrhosis. Nevertheless, the fibrinogen is of a poor quality, "less reactive," and possibly related to the low grade proteolytic activity seen in the plasma of these patients. Some have imputed a deficiency of "thrombin accelerator" to the plasma. Lastly, thrombocytopenia is

frequently a factor, although this patient apparently did not have a significant depression of his platelet levels.

DR. MENDELOFF: In general, when a person with stable chronic liver disease suddenly fails, one suspects either that he is having episodes of hepatic necrosis, or else hepatoma has developed. This latter development is often ushered in by an episode of pain in the general area of the liver, and the liver need not be enlarged. At the same time this cancer may also be associated with complications such as thromboses in the portal system which might well have caused the leukemoid reaction in his blood, the abdominal distention and the gastrointestinal hemorrhages, even though the spleen was not noted to be palpable. He did also have a gallbladder which was not visualized by x-ray; this may have been due to the inability of the liver to excrete the dye, but there is a possibility that acute cholecystitis or acute pancreatitis developed, which might have accounted for his terminal episode.

DR. W. BARRY WOOD, JR.: Could thrombi in the hepatic vein have gotten into the inferior vena cava and involved the renal vein, to explain this very sudden renal insufficiency, or is that unlikely?

DR. MENDELOFF: That has been described, Dr. Wood, but I should have expected him to show hematuria rather than pyuria alone.

DR. REINHARD: If this was portal thrombosis, should not the liver have enlarged terminally, or did he die too soon for that?

DR. MENDELOFF: If the thromboses were in his portal system, they need not have affected the size of the liver.

DR. REINHARD: The clinical diagnoses of the house staff were: (1) Laennec's cirrhosis, (2) acute hepatic necrosis, (3) obstructive emphysema, and (4) acute pyelonephritis, right.

PATHOLOGIC DISCUSSION

DR. DANIEL L. ROSENSTEIN: The body of this man was markedly icteric; there were spider hemangiomas in the skin over the upper thorax and shoulders, and petechiae along the costal margins and about the umbilicus. The testes were atrophic and there was moderate subcutaneous edema about the ankles. The peritoneal cavity contained 5,000 ml. of clear ascitic fluid, and venous varicositis were apparent in the omentum and on the inferior surface of the diaphragm. On cutting through the esophagus some intramural varices were exposed, but these did not distort the mucosa and there was no evidence of any rupture. In the remainder of the gastrointestinal tract there were many petechiae throughout the mucosa, and the contents of the lumen consisted mostly of blood-tinged mucus. A few superficial erosions were present in the fundus of the stomach.

A section of the liver is illustrated in Figure 1. This organ was of decreased size. It weighed 1,160 gm. and was rather soft and flabby. The capsular surface presented numerous rather small and widely separated nodules. On the surfaces exposed by incision there were two types of nodules: a small rust-brown nodule that bulged above the surface, and larger and more irregular nodules which were yellow and soft and had a hyperemic peripheral zone. Between these nodules there were confluent bands of firm, yellow-gray, rather translucent fibrous tissue. The spleen had the appearance of chronic passive congestion and was slightly enlarged to a weight of 320 gm.

The heart was not remarkable except for petechiae and ecchymoses present both in the endocardium and in the epicardium. Petechiae were also scattered over both the visceral and parietal pleurae. The lower lobes of the lungs were atelectatic and slightly congested. The kidneys were scarred and icteric, but their cortices showed no evidence of swelling. The capsular surfaces presented several small cortical cysts and were moderately irregular.

DR. W. STANLEY HARTROFT: The history of this patient, the clinical discussion and the gross findings at autopsy have all served to focus our interest on the liver. Microscopically it is composed of nodules of parenchyma widely separated by bands of a collapsed, rather loosely arranged stroma. (Fig. 2.) Recent cellular necrosis has occurred throughout many of the nodules, with but small zones of surviving parenchyma at their edges in some instances. In the latter regions there is evidence of abortive attempts at regeneration in the form of pseudocholangioles. (Fig. 3.) Hemorrhage is present throughout the necrotic centers of the nodules. Where necrosis has not obscured the picture, it is evident that in many of the nodules portal triads and radicals of central veins are present. Other nodules represent only fragments of liver lobules. Occasional parenchymal cells with more than one nuclei are encountered throughout, but regenerating liver plates, two cells in thickness, are rarely encountered. In the scarred

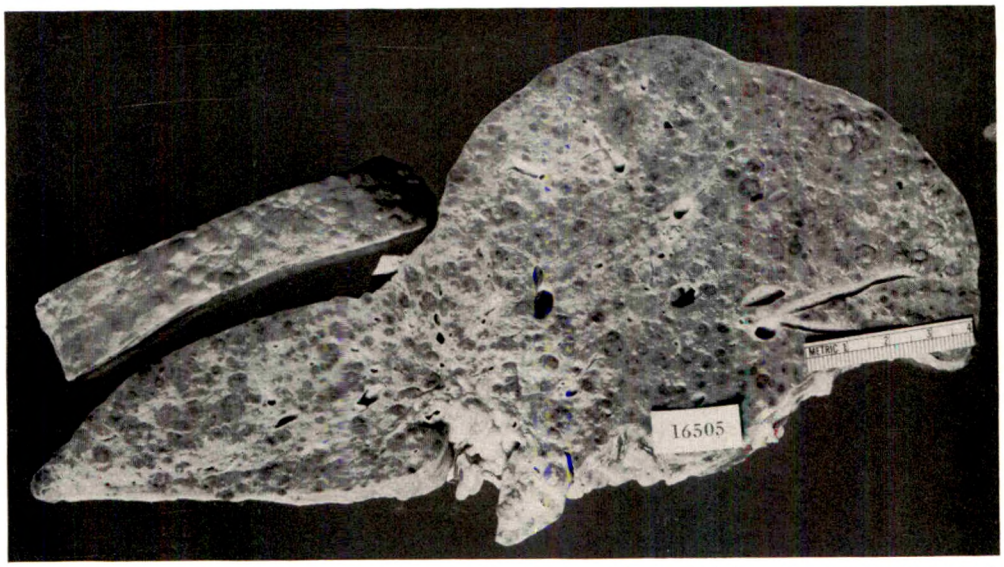


FIG. 1. Sections through the hilum and showing the capsule of the liver. There is extensive interstitial scarring with slightly irregular nodules of surviving parenchyma. The darker nodules are hemorrhagic and necrotic.

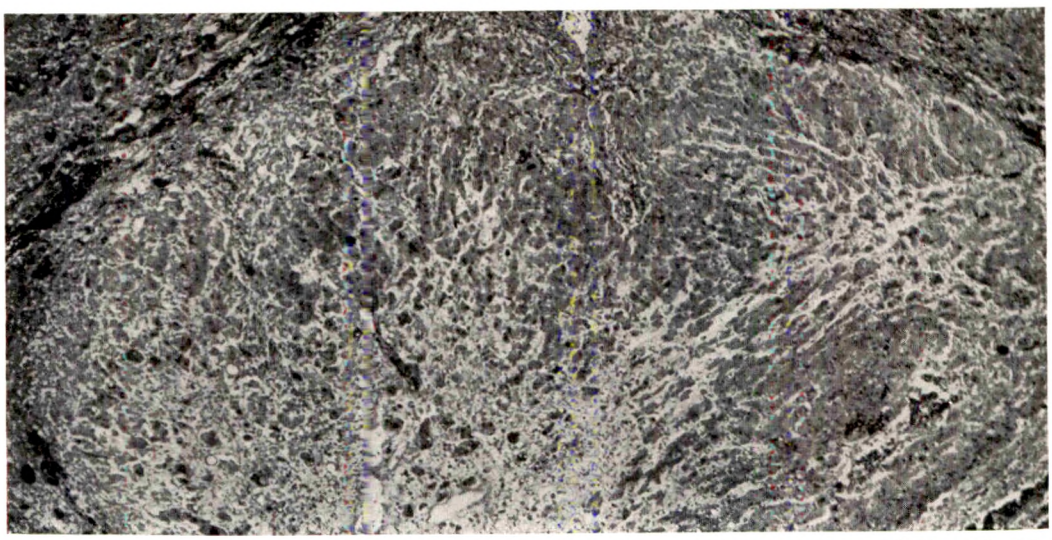
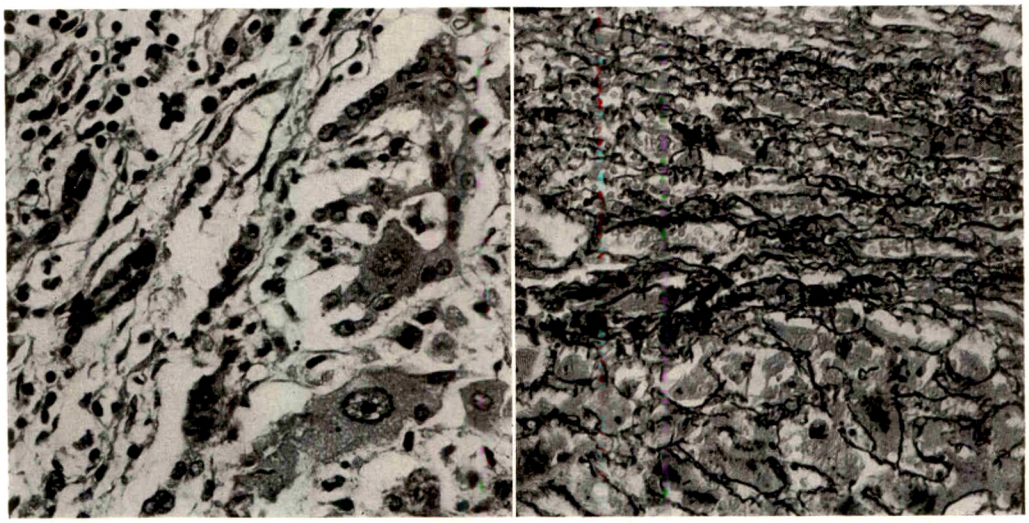


FIG. 2. The area between three nodules in the liver showing the rather loose fibrous scar infiltrated with lymphocytes and mononuclear cells. The parenchymatous nodules contain several portal units and central veins and variable amounts of necrosis.

areas (Fig. 3) generous numbers of mononuclear cells and lymphocytes are encountered. The other cellular constituents of the scarred areas consist of isolated liver cells, cholangioles and blood vessels. By appropriate technics surprisingly little collagen can be demonstrated. Silver stains (Fig. 4), however, reveal that the normal reticulin content of the liver has persisted in the scarred areas, becoming only slightly compressed and contracted, so that the fibers are coarser than usual. Lamination of the reticular fibers is not advanced, still another indication that the adjacent nodules of parenchyma have undergone but little regeneration. Bile pigment is present in many liver cells, and both cholangioles and bile ducts contain plugs of inspissated bile. In this liver, therefore, the parenchymal nodules are multilobular in many instances; many have undergone recent acute necrosis with hemorrhage; and the scarred areas consist chiefly of



3 4

FIG. 3. The edge of a nodule in the liver at high magnification. Pseudocholangioles and lymphocytes are present in the loose scar tissue surrounding the irregular hepatic cells which are arranged in single cell plates.

FIG. 4. Reticulin impregnation demonstrating the slightly coarsened and compressed fibers in the scarred areas in the liver. This change is not nearly as striking as is seen in true cirrhosis.

collapsed, but only slightly compressed reticulin, mononuclear cells, pseudocholangioles and remnants of liver plates. Fat is almost completely absent throughout all sections. The microscopic findings therefore suggest postnecrotic scarring, with a recently superimposed necrosis.

In microscopic sections of the esophagus moderately dilated submucosal veins without evidence of rupture are encountered. In the testes the tubules are atrophic and interstitial cells have disappeared almost entirely. Sections of the kidney reveal evidence of chronic pyelonephritis with considerable destruction of parenchyma. Tubular necrosis is absent.

Finally, in the pancreas portions of the acinous tissue and adjacent fatty tissue appear necrotic. Polymorphonuclear inflammatory reaction is prominent in these areas. In microsections of the heart and the gastrointestinal tract the petechiae seen grossly are visualized, but other significant abnormalities are not encountered.

The gross and microscopic findings revealed at autopsy indicate that the liver had suffered a severe degree of destruction. The histologic features suggest that death of liver cells occurred in at least two distinct episodes. The scarred nodular pattern, absence of fat or evidence to indicate abnormal fat previous to death suggest that the scarring may have been caused by a previous attack of viral hepatitis. The second episode, consisting of acute necrosis in the surviving nodules of parenchyma, is of more recent occurrence, possibly occurring on a circulatory basis. The morphologic evidence fails to suggest that hepatotoxins or nutritional deficiencies were responsible for the production of hepatic damage. It is possible that the development of terminal acute pancreatitis acted as an accessory factor responsible for the final episode of acute hepatic destruction.

Final anatomic diagnoses: Postnecrotic scarring of the liver with acute necrosis of persistent parenchyma; acute pancreatitis with fat necrosis; petechiae and ecchymoses in the gastrointestinal tract, pericardium, urinary tract and skin.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Case Report

Subacute Bacterial Endocarditis Due to Listeria Monocytogenes*

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MEDICAL writings now contain many references to Listeria infection in man. A recent review[1] tabulated 147 cases of reported human infection. Forty-five of these cases were infections of the meninges; none was thought to involve the endocardium. In this same review thirty-six additional cases (nine meningitis, no subacute bacterial endocarditis) were listed which were possibly due to Listeria.

Other reviews[2,3] stress meningitis as being the most frequent form of human Listeria infection. The possible relationship of Listeria to infectious mononucleosis, a human illness in which isolation of Listeria has been reported, was thoroughly discussed by Girard and Murray.[2] No mention was made of subacute bacterial endocarditis due to Listeria in any of these reviews.

In writings dealing with bacterial endocarditis Listeria was not included among the listed etiologic agents.[4–6] So far as we have been able to ascertain, this is the first case of subacute bacterial endocarditis due to Listeria monocytogenes to be reported. Moreover, it is one of the rare instances of human Listeria infection in which significant levels of Listeria agglutinins have been demonstrated.

Adequate descriptions of the history of Listeria, of its bacteriology and epidemiology are contained in any of the reviews previously referred to. Writings pertinent to the immunologic aspects of listerosis are reviewed subsequently.

CASE REPORT

On March 21, 1954, a forty-two year old white man was admitted to the private Medical Service of the Grace-New Haven Community Hospital for study of an illness suspected of being subacute bacterial endocarditis. Three years prior to entry, when he first became the patient of one of the authors (H. M. C.), he was found to have rheumatic heart disease with both mitral and aortic valvular involvement. He could not recall having had rheumatic fever or any of its equivalents although three years prior to this initial examination he had a sore throat with cervical node enlargement. The patient was not seen again until one year prior to admission when he complained of easy fatigability and mild exertional dyspnea. There was no evidence of cardiac failure and the cardiac murmurs were unchanged. An electrocardiogram was normal; by x-ray examination after barium swallow there was slight enlargement of the left auricle. He weighed 203 pounds and was advised to reduce. With restriction of caloric intake, amphetamine and methylcellulose, his weight dropped to 190 pounds, and his symptoms subsided.

In May, 1953, because of persistent pain in his jaws, the patient consulted his dentist. Severe periodontoclasia was discovered, and on June 19, 1953, the patient's remaining twenty-eight teeth were extracted. On the day before, the day of, and the day after surgery, the patient was given 600,000 units aqueous procaine penicillin G intramuscularly. No chills, fever or malaise obtained. Artificial dentures were fitted but were unsatisfactory, for the patient could not chew properly with them. On July 27, 1953, a retained tooth fragment was excised and several sutures were required to close the operative wound. No penicillin was given during this procedure. Four months after this surgery (four months prior to entering the hospital) the patient

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began to have "stiffness and weakness" of both knees. This was most severe on arising in the morning and wore off by evening. Concomitantly, a gradual decrease in "drive and energy" set in. Although the knee pains spontaneously disappeared, the lack of energy grew more prominent as time passed. This he ascribed to poor food intake caused by pain from the false teeth.

On March 6, 1954, he first sought medical attention because of recurring chills and fever. About three weeks before this visit he had an upper respiratory infection manifested by coryza and a non-productive cough. Ten days before, he had a sudden shaking chill, lasting about thirty minutes, followed by drenching sweats and profound weakness. The following day he felt better and went to work. Despite two more episodes of chills and fever, and persistent stiffness of the knees, he continued to work. He had taken no medication except aspirin. When he was examined by one of the authors (H. M. C.) on March 6, 1954, he looked chronically ill. His temperature was 99°F. (orally), blood pressure normal, and heart murmurs unchanged. Despite the absence of characteristic physical findings, the history was such that subacute bacterial endocarditis was suspected and hospitalization was advised. This was refused. The patient returned home and continued to work despite the daily afternoon occurrence of fever and exhaustion. He finally consented to hospitalization for study and treatment on March 21, 1954.

On physical examination at admission the weight was 189 pounds, temperature 99.2°F. (rectal), pulse 88 per minute and respirations 18 per minute. The blood pressure was 140/50 in the right arm with the patient supine. There were no petechiae and there was no rash. The lungs were clear. The heart appeared slightly enlarged by percussion, the rhythm was regular and the aortic and pulmonic second sounds were equal in intensity. A grade 3 harsh, systolic murmur was heard, best in the aortic area, and was transmitted to both sides of the neck. At the apex, the first sound was absent; there was a grade 2 murmur which began late in diastole and extended into systole. No organs were palpable in the abdomen and there was no peripheral edema.

The hemoglobin was 15 gm. per cent and the hematocrit 47 per cent. The erythrocyte sedimentation rate (Wintrobe) was 23 mm. per hour. Total leukocytes were 8,350 per $mm.^3$, with 55 per cent polymorphonuclear forms, 40 per cent lymphocytes, 1 per cent monocytes and 4 per cent eosinophils. A second white blood count forty-eight hours after admission was 10,350. Fasting blood sugar and non-protein nitrogen were normal. Two specimens of urine were normal. Antistreptolysin O was not elevated (125 units). Venous pressure, arm-tongue circulation time and an electrocardiogram were all within normal limits. A culture of the throat grew out only normal flora. There was no growth in any of the six blood cultures taken during the first forty-eight hours in the hospital.

During the four days of this hospital stay the patient was asymptomatic except for a complaint of stiffness of the left elbow. Films of this area revealed no abnormalities. The highest temperature recorded was 100.2°F. (rectal) at 8 P.M. of the day of entry. By radiographic examination there was no abnormality of the paranasal sinuses, and there was no over-all cardiac enlargement. Films of the jaws disclosed a density near the site of the second left maxillary bicuspid, consistent in appearance with a broken root fragment. Since the patient was asymptomatic and no evidence substantiating the diagnosis of endocarditis had been obtained, he was discharged on March 25, 1954. He was advised to keep a daily record of his temperature and to report any fever, or any new clinical development. Several days after discharge the tooth fragment was removed; no pus was found at operation. Prior to and after this surgery he was given 1,000,000 units crystalline penicillin G intramuscularly.

One day after discharge from the hospital the patient noted onset of stiffness in all large joints except the ankles. This persisted for about two weeks and then disappeared. Pain and stiffness reappeared in the knees only three days before re-entry into the hospital. Two weeks prior to readmission the patient noted pain in the pads of the fourth and fifth fingers of the left hand; after one or two days the pain disappeared. During the interval between admissions the patient was unduly tired on returning from work each evening. His appetite fell off. Although there were no chills, the patient felt feverish, usually in the late afternoon, and there were episodes of copious nocturnal hidrosis. At times there was some headache associated with the fever. A sense of imbalance manifested itself by a tendency to bump into doorways. The patient was advised to return to the hospital for re-evaluation but it

was not until April 26, 1954, that he consented to do so.

Physical examination on re-entry disclosed a weight of 190 pounds, temperature of 100.6°F. (rectal), pulse of 88 and respiration of 20 per minute. The blood pressure was 134/64 in the right arm with the patient supine. One petechia was seen in the nail bed of the fourth finger of the left hand. There was no swelling or tenderness of the finger pads. The heart murmurs were definitely changed from those heard at the time of the first admission. There was a grade 3 systolic murmur at the aortic area transmitted to both sides of the neck. A grade 2, blowing diastolic murmur was heard to the right of the sternum in the second intercostal space, and also to the left of the sternum in the third and fourth intercostal spaces. There was a grade 2 systolic murmur at the apex which could be heard well into the left axilla. The spleen was not felt.

During the next two days nine blood cultures were planted and serum was obtained for serologic testing. The hemoglobin was 13 gm. per cent, the hematocrit 42 per cent and the erythrocyte sedimentation rate 42 mm. per hour. Total leukocytes were 9,350 per mm.^{3}, with 75 per cent neutrophils, 18 per cent lymphocytes, 2 per cent monocytes and 4 per cent eosinophils. The urine was normal. A chest film disclosed no significant change from the film taken at the first admission. An electrocardiogram was again normal.

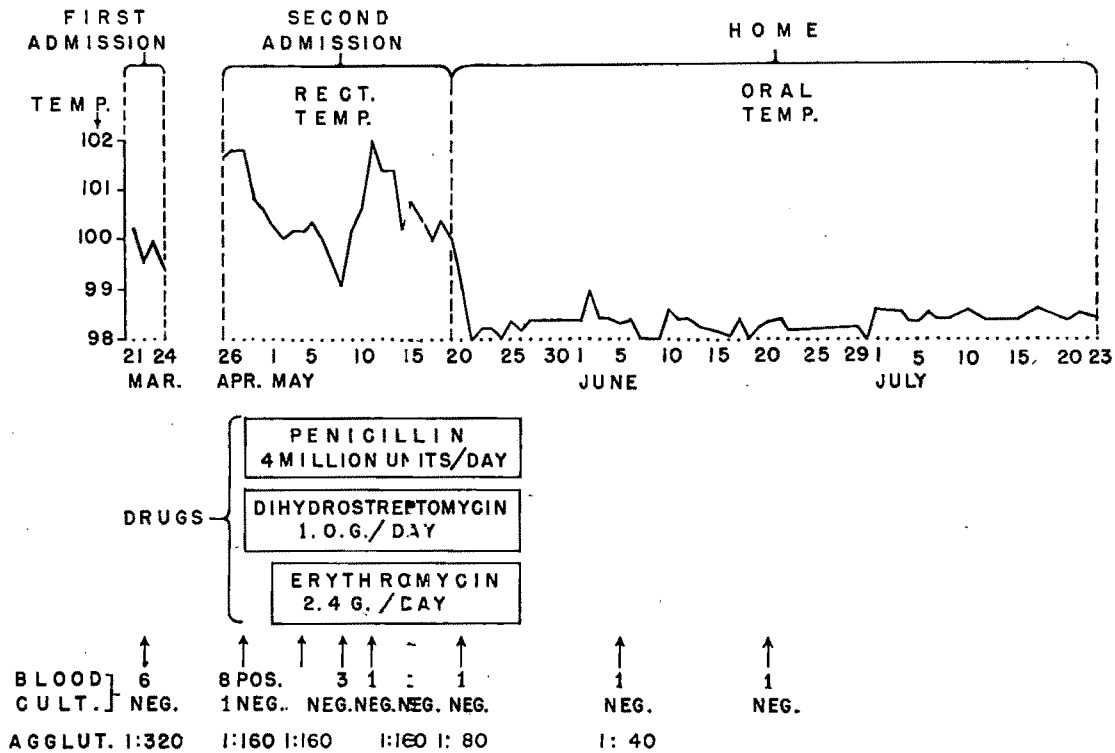


FIG. 1. Summary of clinical course.

Fever persisted and on April 28, 1954, a petechia appeared on the mid-upper lip. The spleen was palpated for the first time on this day, but was not felt again until May 11th. In the belief that the patient had subacute bacterial endocarditis, he was empirically started on a regimen of 1,000,000 units of crystalline penicillin G, intramuscularly, every six hours, and dihydrostreptomycin, 0.5 gm. intramuscularly, every twelve hours. The clinical course has been summarized in Figure 1.

On the following day brucella, typhoid and paratyphoid agglutinations were reported as being negative. Growth was noted in the earliest blood cultures and a gram-positive bacillus was reported. The initial therapeutic regimen was maintained until further identification and sensitivity studies could be completed.

New petechiae were noted on the lips on April 30th. The cardiac murmurs were un-

changed but the temperature was significantly lower. The urine was again free of cells. The results of sensitivity tests run with the bacillus isolated from the blood cultures were reported. (Table 1.) Therapy was altered on May 1st only by the addition of 0.6 gm. of erythromycin, by mouth, every six hours. The temperature curve was definitely swinging downward at this time.

Erythematous, pruritic wheals appeared on the skin of the wrists, thighs and elbows on May 4th. No new petechiae were found. Administration of antihistamine medication gave some relief from itching. On May 6th, since new wheals were still appearing, penicillin G was discontinued and crystalline penicillin O was substituted in the same dosage. The urine was clear on May 6th but on May 7th there were three to four red cells, and a rare white cell per high power field. By May 8th the wrist lesions were subsiding, but the lower leg lesions were red, confluent, 5 by 10 cm. patches, which were slightly pruritic. There were no red cells in the urine at this time and none were found subsequently.

On May 9th dull, steady, lower left chest pain was noted. Physical examination revealed no new findings. During the next two days the chest pain persisted but assumed a pleuritic character, and was accompanied by left upper quadrant abdominal pain. At this time, May 11th, the hemoglobin was 14 gm. per cent, white count 19,250 with 78 per cent neutrophils, 17 per cent lymphocytes, 4 per cent monocytes and 1 per cent eosinophils. Late evening elevation of temperature accompanied the onset of the chest pain; by May 11th the temperature remained elevated throughout the day, attaining a maximum of 102°F. Chest films were read as showing left lower lobe pulmonary infarction and definite cardiomegaly. An abdominal film disclosed one dilated loop of small bowel but there were no physical findings suggestive of mesenteric thrombosis. At this time there was a decrease in breath sounds and tactile fremitus over the left lower chest, and a tender, palpable spleen. In the legs there was no venous distention, no change in skin temperature and no abnormal consistency or tenderness in the calf muscles. The stool was guaiac-negative.

By May 14th the temperature had fallen to 100°F. Pain and signs persisted in the chest but the spleen was no longer palpable. On May 17th the chest pain was much decreased. The left diaphragm was still elevated by radiography but the density had markedly cleared. Both breath sounds and tactile fremitus were still decreased at the left base. The hemoglobin was 13 gm. per cent, white count 17,650, with 64 per cent neutrophils, 25 per cent lymphocytes, 7 per cent monocytes and 4 per cent eosinophils.

TABLE 1

SENSITIVITY OF LISTERIA MONOCYTOGENES, STRAIN ISOLATED FROM PATIENT IN THIS REPORT TO VARIOUS ANTIBACTERIAL AGENTS

Agents	Concentration	Result
Single:		
Sulfathiazole	1 mg./ml.	Resistant
Sulfadiazine	1 mg./ml.	Resistant
Sulfamerazine	1 mg./ml.	Resistant
Bacitracin	20 μ/ml.	Slightly sensitive
Chlortetracycline	10 μg./ml.	Very sensitive
Oxytetracycline	10 μg./ml.	Very sensitive
Tetracycline	10 μg./ml.	Very sensitive
Chloramphenicol	10 μg./ml.	Very sensitive
Erythromycin	1 μg./ml.	Very sensitive
Streptomycin	10 μg./ml.	Moderately sensitive
	100 μg./ml.	Very sensitive
Penicillin	1.5 μ/ml.	Resistant
	10.0 μ/ml.	Very sensitive
Combined:		
Penicillin	1.5 μ/ml.	Very sensitive
Streptomycin	10 μg./ml.	
Penicillin	1.5 μ/ml.	Resistant
Sulfisoxazole	1 mg./ml.	
Penicillin	1.5 μ/ml.	Very sensitive
Oxytetracycline	10 μg./ml.	
Penicillin	1.5 μ/ml.	Very sensitive
Chloramphenicol	10 μg./ml.	
Streptomycin	10 μg./ml.	Very sensitive
Chlortetracycline	10 μg./ml.	
Streptomycin	10 μg./ml.	Very sensitive
Oxytetracycline	10 μg./ml.	
Streptomycin	10 μg./ml.	Slightly sensitive
Chloramphenicol	10 μg./ml.	

Pain and signs had disappeared from the chest by May 19th. The temperature hovered around 100°F., an elevation thought consistent with the patient's demonstrated penicillin hypersensitivity. The patient was allowed to go home on this day, to continue one more week on all three antibiotics in the same dosage as given in the hospital. He was instructed to measure his temperature daily and keep a record of the readings. When seen at home during this final week of antibiotic therapy he was found to have remained afebrile and asymptomatic.

On June 4th, ten days after cessation of antibiotic therapy, a blood culture showed no growth. At that time, the hemoglobin was 13 gm. per cent, hematocrit 42.5 per cent, white count 12,350, with 53 per cent neutrophils, 33 per cent lymphocytes, 5 per cent monocytes, 8 per cent eosinophils and 1 per cent basophils. Two

weeks later a blood culture again showed no growth.

When examined on July 8th the patient felt quite well. His record of temperature readings disclosed no fever during the six-weeks' period following discontinuance of antibiotic therapy. There was no dyspnea on moderate exertion, and the patient was eager to go back to work. The cardiac findings remained as previously described and were the only abnormality on physical examination. An electrocardiogram was again normal. Re-examinations in the months of August, September and October failed to reveal any evidence of clinical relapse.

Bacteriologic Studies. Eight of the nine blood cultures drawn during the first forty-eight hours of the second hospital admission grew out gram-positive bacilli. Cultures taken on May 7th (No. 10, 11, 12), May 11th (No. 13), May 13th (No. 14), May 19th (No. 15), June 4th (No. 16) and June 18th (No. 17) were all sterile.

This organism grew readily on the usual laboratory media. Hemolysis occurred on blood agar, but took two to four days to appear on initial isolation. The bacillus was motile, both by hanging drop inspection and by growth in semi-solid medium. When offered various sugars, no gas was formed but acid was produced from: glucose, lactose, sucrose (late), maltose, mannitol, salicin, xylose and rhamnose. No reaction occurred with inulin, inosite, dulcite or arabinose. Citrate was not utilized, indole was not produced and H_2S was not formed. Urea was split.

Forty-eight hours after inoculation of the conjunctiva of an adult albino rabbit by swabbing gently with a twenty-four-hour broth culture, there followed a purulent conjunctivitis.[7,8] By microscopic examination, gram-positive bacilli could be seen within the leukocytes of the exudate. Culture of this pus grew out only gram-positive bacilli.

An adult albino mouse was given, by intraperitoneal injection, 0.5 ml. of a twenty-four-hour broth culture. This mouse died after ninety-six hours. At necropsy the liver, spleen and mesentery were studded with small, pinhead sized, white nodules. Cultures of these nodules, peritoneal exudate, heart blood, all grew out gram-positive bacilli in pure culture.

This combination of morphology, cultural characteristics, biochemical reactions and pathogenicity is sufficiently unique to identify this organism as Listeria monocytogenes.

Agglutination Studies. Using live, twenty-four-hour cultures of the Listeria isolated from the patient (grown in trypticase-soy medium at 37°C.), the patient's serum was tested for agglutinins. Serum was diluted with sterile saline to which had been added crystalline penicillin G (final concentration 10 units per ml.) and streptomycin (final concentration 25 μg. per ml.). To 0.5 ml. amounts of each serum dilution, in 10 by 75 mm. tubes, was added 0.5 ml. of the culture. The tubes were capped with parafilm, mixed by repeated inversion, and incubated at 37°C. for two hours. After twenty-four hours in the refrigerator they were read. Firm edges to the agglutinated button, together with a clear supernatant, were taken as evidence of complete agglutination (designated C in Table II). Slight turbidity, as compared with the saline control, a diffuse edge to the precipitate, and coherent masses spinning up on tapping the tube, were taken as signifying incomplete agglutination (designated I in Table II). Lesser degrees of agglutination were considered too subjective in reading to be worthy of reporting (designated 0 in Table II). Agglutinations were attempted using formalinized bacteria. A twenty-four-hour broth culture was treated with 1/50 volume of 37 per cent formalin by incubation at 37°C. for twenty-four hours; the bacteria were washed twice with normal saline, re-suspended in normal saline for use. With this antigen, agglutination was observed in all tubes, including the saline control.

TABLE II
AGGLUTINATION OF LISTERIA ISOLATED FROM THE PATIENT BY THE PATIENT'S OWN SERUM (SEE TEXT FOR METHODOLOGY)

Date	Final Dilution								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Saline
March 22, 1954	C	C	C	C	C	C	I	0	0
April 27, 1954	C	C	C	C	C	I	0	0	0
May 4, 1954	C	C	C	C	C	I	0	0	0
May 13, 1954	C	C	C	C	C	I	0	0	0
May 19, 1954	C	C	C	C	I	0	0	0	0
June 4, 1954	C	C	C	I	0	0	0	0	0
Control*	C	I	0	0	0	0	0	0	0
Normal†	0	0	0	0	0	0	0	0	0

C = complete agglutination; I = definite, but incomplete agglutination; 0 = no definite agglutination.

* Control serum obtained from a patient in first week of therapy for Strep. viridans subacute bacterial endocarditis.

† Normal serum obtained from one of the authors (P. H.).

Control serum was obtained from a patient ill with subacute bacterial endocarditis due to Streptococcus viridans; this patient was in her first week of therapy. Normal serum was obtained from one of the authors (P. D. H.). The agglutination results are tabulated in Table II; serum from the first admission (March 22nd) was also tested for agglutinins.

COMMENTS

One can only speculate as to when this patient's illness began. From the agglutination and historical data he must have been ill for some time before his admission on March 21, 1954. He was known to have had occasional episodes of fever for at least three weeks prior to this admission. He had experienced arthralgia and asthenia four months before this admission and it is possible that his disease began at this time. Since subacute bacterial endocarditis frequently follows dental surgery and usually has an insidious onset, it is not impossible that the disease began as far back as the dental surgery of June and July, 1953. In favor of a shorter duration of illness was the finding at the initial admission of a normal hemogram, and the development during the interval between admissions of the anemia characteristic of subacute bacterial endocarditis. As far as could be ascertained, the patient had no contact with animals that might have been infected with Listeria.

The source of the embolus causing infarction of the left lower lobe of the lung could not be definitely fixed. There was no clinical evidence of leg vein thrombophlebitis. While there were no definite signs of tricuspid or pulmonary valve disease, involvement of these valves might have been masked by murmurs arising from the already diseased aortic and mitral valves.

Urticaria, due to allergy to penicillin G, was the only untoward effect engendered by the antibiotic therapy given this patient. By substituting penicillin O for the offending penicillin G, and giving antihistamine medication, it was possible to continue to give large doses of penicillin with minimal discomfort to the patient.

Of most interest in this case is the finding in the patient's serum of a significant level of agglutinins for the Listeria causing his illness. It should be emphasized that except for two reports, to be discussed, studies on immunity in human Listeria infections have failed to reveal significant antibody levels.

Janeway and Dammin[9] pointed out the Listeria agglutinating power of horse serum. In serum from a patient acutely ill with Listeria meningitis, Carey[10] reported Listeria agglutination in a dilution of 1:640. Because this patient had received anti-meningococcus horse serum, and since there were no agglutinins in serum obtained two weeks after recovery, the initial high titer probably represented passively obtained horse serum agglutinins.

Kapsenberg[11] reported a titer of 1:800 in a patient ill with Listeria meningitis. No information was given as to time during illness when this serum was obtained, or what preceding and succeeding titers were. The patient survived and it was mentioned that sulfapyridine was used in treatment; no statement as to whether or not horse serum had been given was included. Further, there was no description of the agglutination method and no mention was made of control sera.

Beute et al.[12] described listerosis in a sixty-nine year old woman. This illness apparently began as a severe conjunctivitis, with signs and symptoms of meningitis developing about a week later. Listeria was isolated from the cerebrospinal fluid. Sera from the patient were tested for agglutinins active against the Listeria isolated, with these results:

1 week after onset of conjunctivitis (4 days after meningitis)	negative
3 weeks after onset of conjunctivitis (17 days after meningitis)	1:1600
5 weeks after onset of conjunctivitis (32 days after meningitis)	1:800

Penicillin and sulfonamide therapy secured recovery; there was no mention of horse serum administration. The agglutination method was not described nor was use of control sera.

Bennett et al.[13] in reporting a case of Listeria meningitis sought serum agglutinins against the infecting Listeria. No agglutinins were found in sera obtained from the twelfth to the thirty-fifth hospital day. Both live and formalinized bacteria were used.

Line and Cherry[14] described two cases of Listeria meningitis. Using formalinized bacteria, complete agglutination was not obtained beyond a final dilution of 1:50; a trace was reported at a final dilution of 1:200.

Finegold et al.[15] looked for agglutinins in one of their two cases of Listeria meningitis. They obtained only partial agglutination at a final

dilution of 1:40 with acute serum and 1:80 with convalescent serum. Formalinized bacteria were used in their studies.

From these data it seems most likely that significant agglutination titers are not associated with Listeria meningitis. Infectious mononucleosis, according to Webb's case,[16] also does not make for high agglutinin titers. He reported a classic case of infectious mononucleosis (heterophile titer of 1:2048); Listeria was isolated from the blood. This patient's serum gave only a trace agglutination at a 1:25 dilution on the thirty-seventh day after onset of the illness. One year later the same titer was obtained.

Janeway and Dammin[9] found no significant quantity of agglutinin for Listeria in the sera of thirteen patients ill with indisputable infectious mononucleosis.

The finding, then, of subacute bacterial endocarditis due to Listeria, while in itself unique, presents one of the few instances of Listeria infection in which significant antibody production has been demonstrated. Just as the non-invasive character of Listeria might lead one to predict it would be found to cause subacute bacterial endocarditis, so might one also predict that in such an infection significant levels of antibody would obtain, as the allergic aspects of subacute bacterial endocarditis pathogenesis demand.[17–19]

SUMMARY

1. A case of subacute bacterial endocarditis due to Listeria monocytogenes is described. A review of the writings on both human Listeria infections and bacterial endocarditis has disclosed no previously reported case of subacute bacterial endocarditis due to this organism.

2. Significant levels of agglutinins active against the Listeria causing the patient's illness were demonstrated in his serum. The agglutinin titer fell from 1:320 in the untreated state to 1:40 after cure. In more common forms of human Listeria infection significant agglutinin titers have rarely been reported.

3. Antibacterial therapy consisted of concomitant administration of crystalline penicillin, dihydrostreptomycin and erythromycin. Five months after cessation of therapy, there was no indication of relapse.

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The American Journal of Medicine

VOL. XIX OCTOBER, 1955 NO. 4

Editorial

The Interrelationships of Ventilation, Circulation and Metabolism

COMPARED to the vast amount of investigation of circulatory mechanics little attention has been given to the ventilatory counterpart of the homeostatic mechanism. Much recent work has been done in the laboratory of the physiologist to remedy this defect but so far little of it has permeated clinical thought.

The superficial aspects of the ventilatory act have long been familiar to clinicians; such aspects include the types of breathing described by Kussmaul, Cheyne-Stokes and Biot, the orthopnea of heart failure or asthma and the rapid shallow breathing of pneumonia. Interspersed in the clinical jargon of the day frequent surmises can be heard that this or that anxious or hysterical person exhibits a "hyperventilation syndrome," yet rarely is an effort made to verify the conjecture even by testing Chvostek's sign. This is understandable as regards laboratory confirmation because of the ephemeral nature of the phenomena and the elaborate equipment requisite for the proof. Hypoventilatory syndromes with respiratory acidosis are recognizable in morphine poisoning, emphysema with bronchitis or bronchitic asthma or in some cases of poliomyelitis, and the clinical surmise can be checked by determinations of the pH and tensions of the respiratory gases in the arterial blood. It was not easy to recognize that in many patients with poliomyelitis hyperventilation occurred in the respirators and this was often the cause of the subsequent difficulty in weaning them from the "iron lung." This is apparently due to the increased sensitivity which the respiratory center acquires as a part of its adaptation to low carbon dioxide tensions resulting from prolonged hyperventilation.

It is with the aberrations of homeostasis that the clinician is primarily concerned. To maintain constancy of the internal environment of the body, in view of the wide variations in the metabolic demands of the cells, requires the closest coordination of the ventilatory events with those taking place in the circulation. Disturbance of the normal relationships between ventilation and blood flow is immediately reflected in the tensions of the alveolar gases and their equilibration with those of the blood. Unless these changes are promptly compensated for by electrolyte shifts between plasma and cells, the degree of alkalinity of the blood may be altered until a slower form of compensation is effected, primarily by the activity of the kidneys and also by the gastroenteric mechanisms for altering the base, chloride and phosphate of the blood. Conversely, when the primary disturbance occurs in these latter mechanisms or through some alteration in the intermediary metabolism, the most prompt reactions toward homeostasis are those affected by altering ventilation and blood flow, assuming, of course, that the coordinating activity of the nervous system is not impaired by narcosis, blocking agents or other pharmacodynamic effects which may alter the sensitivity of the nervous system mechanism.

In recent years an enormous amount of work has gone into the systematic mapping out of the afferent nervous system pathways from various

types of receptors distributed widely over the body, by means of which the modifications of ventilation and circulation are mediated. These receptors are of two general types, those which are stimulated mechanically and are known as "stretch receptors," and those which are stimulated chemically by changes in reaction or changes in CO_2 and O_2 tension and are known as "chemo-receptors." The progress of this work has been well reviewed recently by Dawes and Comroe.[1]

The mechanics of respiration was the subject of a review by W. O. Fenn in this journal in 1951.[2] A valuable compilation of the many papers from Fenn's laboratories was published in a technical report of the United States Air Force in the same year.[3] Rahn[4] in one of these papers (and Riley and Cournand[5] independently) deals very clearly with the relationship of ventilation to blood flow in the pulmonary gas exchange of normal subjects. While this implies normal regulatory mechanisms and no abnormal barriers to diffusion of gases between the alveoli and the pulmonary capillaries, such as may exist under pathologic conditions, these papers will remain important guides to clinical thinking when dealing with such conditions. Rahn has derived an equation from the blood flow equation of Fick and the alveolar ventilation equation of Fenn. With the aid of the Henderson diagram for blood and the Fenn diagram for gas respiratory quotient, Rahn was able to represent graphically all the simultaneous tensions of oxygen and carbon dioxide which satisfy the combined equation in which the use of alveolar ventilation V_a and blood flow F as a ratio, V_a/F, makes it unnecessary to take account of the level of metabolism. Each point on the curve represents a particular V_a/F ratio with its corresponding respiratory quotient. At the usual or average respiratory quotient of 0.8 the V_a/F ratio is 1.0 and the corresponding partial pressures of O_2 and CO_2 are 100 and 40 mm. Hg respectively. The mean of the normal range of alveolar respiratory quotients is from 0.7 to 1.0; with mean values above 1.0 the V_a/F ratios are increased and relative hyperventilation is indicated, while respiratory quotients below 0.7 and correspondingly low V_a/F ratios indicate relative hypoventilation.

In clinical thinking the concept of this ratio becomes important in that hyperventilation may be dependent as much upon failure of the blood flow to maintain parity with the ventilation as upon augmentation of the ventilation *per se*. Conversely, hypoventilation may depend as much upon an increased blood flow with which the ventilation does not maintain parity as upon depression of the ventilation.

In a recent paper by Alexander, Spalter and West[6] the hyperventilation of salicylate toxicity has been shown to be due to increased respiratory sensitivity to the carbon dioxide stimulus. In this issue of the Journal, Tenney and Miller[7] have studied the circulatory, ventilatory and metabolic effects of this drug. They, too, have found that salicylate exerts a direct respiratory stimulant action on the medulla, independent of aortic or carotid chemoreceptors. They have added that salicylate is a profound metabolic stimulant acting primarily on skeletal muscle. The circulatory response to salicylate is affected partially by an increase in cardiac output and to a large extent by a widening of the A-V oxygen difference. The net result was a lowering of the $P_A\ CO_2$ and a rise in pH. In other words, the V_a/F ratio was increased by the failure of the cardiac output to increase in proportion to the rise in ventilation and metabolism.

In a previous paper[8] Tenney pointed out that the diminished response of the respiratory center of a patient with emphysema is proportional to the degree of CO_2 retention. In general, he finds that the sensitivity of the respiratory center, as indicated by the slope of a stimulus response curve, appears to change in a manner inversely proportional to the degree of CO_2 retention.

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In Tenney's studies on salicylate an interesting and important difference was found when oral administration is compared with intravenous injection of the drug. With oral administration the respiration is stimulated but the increase in the sensitivity of the center does not occur until the kidneys have had time to excrete base and lower the bicarbonate level of the plasma. Thus the person who receives salicylate by mouth reacts as does the person who is adapting himself to the hypoxia of high altitude. When, on the other hand, salicylate is given intravenously the respiration is stimulated to the production of an alkalosis, yet the center is not rendered more sensitive because there is no immediate compensatory lowering of the bicarbonate level, and the metabolic effect of the drug has greatly increased CO_2 production.

These experiments of Tenney illustrate beautifully the precise correlation that must exist between ventilation, blood flow and metabolism if homeostasis is to be maintained.

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Clinical Studies

The Respiratory and Circulatory Actions of Salicylate*

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THE voluminous literature on the salicylates attests not only to their importance but also to the uncertainties and controversies associated with their action. Comprehensive reviews[1–3] over the years have emphasized many of the areas of doubt. Most authors agree that respiratory stimulation is an important action of the salicylates but precise quantitation of this response is wanting. The locus of action is not certain, although there are reports in the literature which purport to show that peripheral receptor systems are essential if stimulation is to occur.[4–7] Direct medullary stimulation has been implied in many instances but remains unproven. The important question of salicylate effect on the acid-base balance of the body still remains controversial, although there is accumulating evidence which indicates that respiratory alkalosis is the most common result in adult man.[8] Arguments persist as to whether there is a transient period of acidosis which serves as the initial respiratory stimulant. The effect of salicylate on gaseous metabolism[9,10] has been largely neglected and may serve as a major clue to the final effects on both the respiratory response and the ultimate acid-base pattern. Finally, the response of the circulatory system, which must serve as the integrator between lung and tissue in the altered respiratory state of salicylism, has not been adequately studied.

Experiments (designed for both man and dog) were performed to answer some of these problems and they form the subject matter of this report.

METHODS

Mongrel dogs anesthetized with intravenous sodium pentobarbital (27 mg./kg. body weight) constituted the principal experimental preparation. Attention was directed to maintaining as nearly as practicable a steady state under anesthesia. This was done by continuing an intravenous drip of low concentration pentobarbital in saline solution (half of the calculated anesthetic dose in 500 cc. saline solution) at the rate of approximately 40 drops per minute. An endotracheal tube was inserted and fitted to a low resistance check valve. Alveolar (end tidal) samples were collected by syringe from an endotracheal polyethylene catheter. One ml. aliquots were aspirated at end tidal flow for five successive breaths and analyzed for CO_2 and O_2 by means of the Scholander-Roughton micro-gas analyzer.[11] Arterial blood was collected from the carotid artery and mixed venous blood from the right ventricle via catheter. Blood samples were analyzed for CO_2 and O_2 contents by the method of Van Slyke and Neill.[12] Whole blood pH was determined immediately at 38°C. by the Beckmann model G pH-meter. Intravascular pressures were recorded with Statham strain gauges and a Sanborn polyviso oscillograph.

All observations on man were made while the subject lay comfortably in the supine position. Oxygen consumption was determined under basal conditions. Ventilation measurements were made through a mouthpiece and check-valve arrangement, the input of which led from a Douglas bag containing the desired inspiratory gas mixture and the outlet connected to a low resistance gas meter previously calibrated at both high and low flow velocities against a spirometer. Respiratory frequency was recorded with pneumograph. The inspired gas mixtures employed were 100 per cent O_2, 3 per cent CO_2 in 97 per cent O_2, and 5 per cent CO_2 in 95 per cent O_2. These mixtures were breathed for

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fifteen minutes each but the mean minute volume and respiratory frequency for only the last five minutes were computed. All volumes were corrected to body temperature and pressure, saturated with water vapor (BTPS). At the end of each run a Haldane-Priestley alveolar air sample was collected and analyzed for CO_2 and O_2. The respiratory dead space was measured in each subject by determining the alveolar and expired CO_2 concentrations and applying the Bohr equation. Alveolar ventilation was then calculated in the usual way [alveolar ventilation = minute volume expired − (respiratory frequency × dead space volume)]. The normal subjects were all trained in respiratory maneuvers, and at least three consistent control runs were required before evaluating effects of the drug. Acute experiments involving intravenous salicylate were preceded by a control run on all gas mixtures immediately prior to exhibition of a drug. The salicylate response was observed by initiating measurements twenty minutes after drug administration.

Sodium salicylate solution was always employed in a concentration of 1 gm. per 5 cc., pH 3.5.

Patients on salicylate therapy were studied in the same manner except that no data were obtained on CO_2 breathing.

RESULTS

Acute Response to Sodium Salicylate in the Dog. Figure 1 illustrates a representative experiment in which the immediate changes following the intravenous injection of sodium salicylate (100 mg./kg. body weight) are recorded. Following an approximately one-minute latent period, ventilation was enhanced and the alveolar CO_2 tension began to fall. The only detectable change in arterial pH was a continuing rise from the normal value concomitant with the fall in CO_2 tension. No early fall in pH preceding respiratory stimulation was ever observed. Beginning shortly after the initial increase in ventilation there developed a gradual increase in oxygen consumption which reached its maximum in about twenty-five minutes. At this dosage range the oxygen consumption was nearly doubled. The early hyperventilation was attended by an increase in the respiratory exchange ratio but by the time oxygen consumption had reached its maximum the respiratory quotient had returned to control values, and the oxygen consumption and carbon dioxide production were equivalent. That the increase in oxygen consumption is not secondary to the increase in ventilation will be shown subsequently. Indeed, the major part of the increase in ventilation after the first five minutes of the effect of the drug is secondary to the augmented CO_2

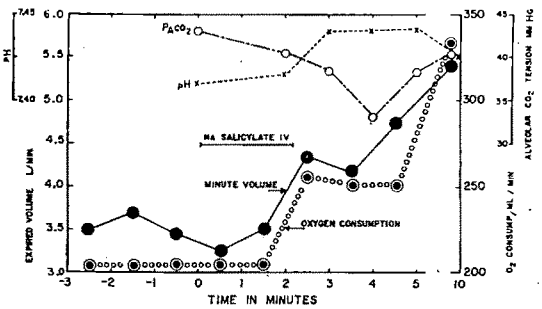


FIG. 1. Respiratory responses of the dog immediately following the intravenous injection of 100 mg./kg. body weight sodium salicylate.

production. It is for this reason that the later increase in ventilation is not attended by much further fall in alveolar CO_2 tension or further rise in arterial pH. In some instances this metabolic demand on ventilation may exceed its response, particularly under deep anesthesia, and a rise in CO_2 tension in the blood and alveolar air may result. Some of the questions which such observations pose are considered in the following experiments.

Metabolic Effect of Salicylate. The pronounced effect of salicylate on respiratory gas metabolism is unequivocal. Figure 2 illustrates the dosage-response relationship of this effect. Apparently there is a maximum change in oxygen consumption achieved at about 100 mg./kg. for sodium salicylate. At higher levels the range of response is wide; at very high doses (150 mg./kg.) many individuals may respond less than with a smaller dose.

The source of such a major metabolic effect is probably in skeletal muscle. The predicted response to a dose of 100 mg./kg. is achieved in both the eviscerated dog and the "functionally hepatectomized" dog (Eck's fistula with hepatic artery ligation), indicating that the liver is not the principal source of increased oxidative requirement. Figure 3 illustrates that the response is independent of central and peripheral nervous control. Three decapitated dogs revealed the predicted metabolic response to salicylate; a similar number under the influence of curare showed no difference in this response

from intact dogs. The metabolic response is also readily exhibited in the presence of established adrenal blockade (dibenzyline®) so that the "calorigenic action" of epinephrine is not implicated. This would appear to establish that the response reflects metabolic processes in the

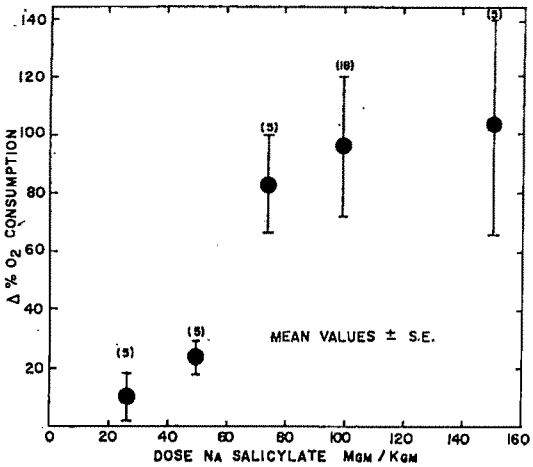


FIG. 2. Dose-response data for sodium salicylate effect on oxygen consumption in the dog; determinations made twenty-five minutes after drug administration. Numbers in parentheses refer to number of experiments performed to define each mean point.

peripheral tissues. Recent *in vitro* studies support this contention,[13] although Lutwak-Mann could detect no significant change in the oxygen consumption of liver slices except in the presence of high concentrations of salicylate when it acted as a metabolic depressant.[14]

The respiratory response to salicylate in the intact dog is a summation of (1) direct central stimulant action and (2) increased metabolic production of carbon dioxide. The magnitude of the latter stimulus may be seen in isolation if ventilation is held constant throughout the experiment. Figure 4 illustrates a typical experiment in which salicylate was administered to a dog already under the influence of curare, and in which ventilation was artificially maintained at a constant rate in a whole body respirator. As the metabolic effect develops the blood reaction becomes acid and the tension of carbon dioxide rises in the alveolar air. The extent of this acute respiratory acidosis is directly proportional to the increase in metabolic CO_2 production. It is clear from these experiments that the increase in oxygen consumption observed in the intact dog is not secondary to hyperventilation.

Salicylates as Direct Central Nervous Stimulants. The observed fall in alveolar CO_2 tension and

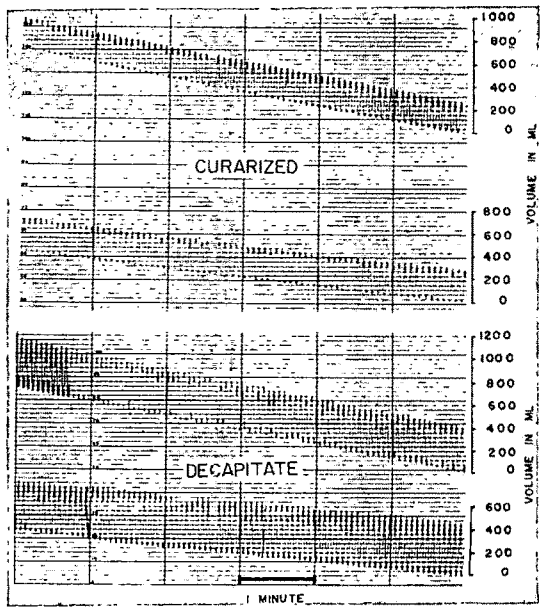


FIG. 3. Segments of spirometer tracings of decapitated and curarized dogs maintained at constant ventilation in whole body respirator. In each pair the lower trace is control and the upper is the record made twenty-five minutes after salicylate (100 mg./kg.).

rise in arterial pH in the intact dog following salicylate administration implies a direct action on the ventilatory mechanism. Experiments have been interpreted as proving that this effect is mediated via peripheral receptors since it could not be demonstrated in the vagotomized animal.[4,5,6,7] It should be emphasized, however, that in each instance two large salicylate doses were administered within a short time interval, once before and once following vagotomy. Lack of ventilatory response to the second dose cannot be accepted as final evidence for the necessity of vagal pathways since the same lack of response is seen in the intact dog when a second dose closely follows the first.

If the major part of the ventilatory increase is secondary to the metabolic stimulating action of salicylate, it would be anticipated that the medullary centers only would be involved since this is the locus of action for CO_2. The direct drug stimulating effect might, however, depend upon a different area. That this area is not the chemoreceptor of the carotid or aortic arteries is shown by experiments of the type illustrated in Figure 5. Both vagi were severed and both carotid bodies denervated in the dog. Functional denervation was established since no respiratory stimulation occurred when the

animals breathed 5 per cent O_2 in 95 per cent N_2. However, immediately after administration of sodium salicylate intravenously there was an increase in the minute volume expired. The subsequent sequence of events in the vagotomized animal differed from the intact preparation

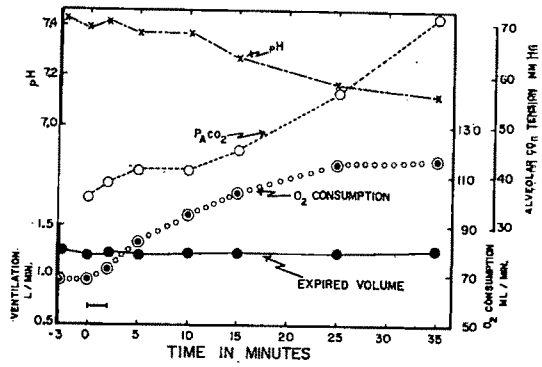


FIG. 4. Effect of 100 mg./kg. sodium salicylate on acid-base balance and respiratory gas metabolism of curarized dog in whole body respirator maintained at constant ventilation; drug administered at zero time over two minute time interval.

only in that there was less increase in respiratory frequency.

That the locus of action of direct salicylate respiratory stimulation is in the central nervous system may be shown by applying low concentrations of salicylate to the region of the medulla.

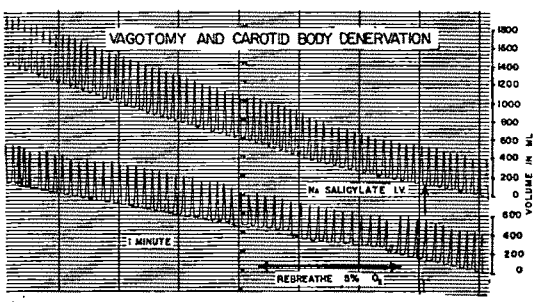


FIG. 5. Spirometer record of dog with both vagi sectioned and both carotid bodies denervated; ventilation is not altered by 5 per cent O_2 in 95 per cent N_2 but increases after intravenous injection of sodium salicylate (100 mg./kg.).

Three dogs studied in this manner gave consistent results, and a representative experiment is noted in the graphs in Figure 6. A needle was placed in the cisterna magna with the dog maintained in a slightly head-down position. Control observations were afforded by slowly withdrawing 2 ml. of cerebrospinal fluid and replacing an equal volume of phosphate buffer at pH = 7.34. Minimal changes in ventilation occurred. Again 2 ml. were withdrawn, and a solution of sodium salicylate (1:500) in phosphate buffer adjusted to pH = 7.34 was injected. This was followed by a prompt and dramatic increase in ventilation, a fall in alveolar CO_2 tension and a rise in arterial pH, which oc-

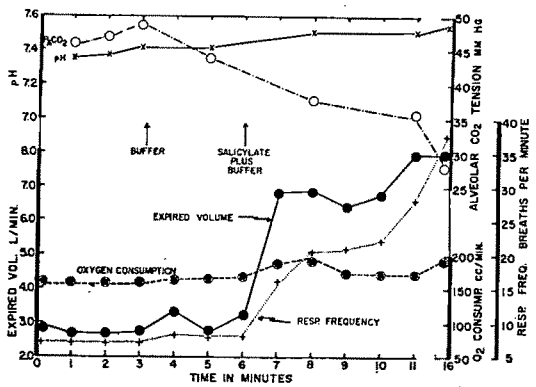


FIG. 6. Respiratory changes following injection of 2 cc. 1:500 sodium salicylate in buffer into cisterna magna of dog; see text.

curred without any attendant rise in oxygen consumption. These effects then represent only the direct stimulating action of salicylate on the central nervous system. It is of considerable interest that as this action became more marked the respiratory frequency increased to such a degree that dead space ventilation occupied a larger fraction of the minute volume and effective alveolar ventilation remained essentially constant in spite of the continuing increase in minute volume expired. It is for this reason that the alveolar CO_2 tension and arterial pH levelled off after the first few minutes, but at a new level characteristic of respiratory alkalosis.

TABLE I
PHYSICAL DATA OF NORMAL SUBJECTS

Subject	Age, Sex	Height (cm.)	Weight (kg.)	Surface Area (M^2)	Dead Space Volume (cc.)
R. M.	34, M	183	77	2.0	118
A. M.	26, F	174	59	1.72	114
B. S.	27, M	186.5	95	2.2	100
M. C.	28, F	171	60	1.7	111
M. T.	32, M	176	68	1.85	160

Salicylate Action in Normal Man. Five normal adult subjects with physical characteristics cited in Table I were studied in the manner described

under the section on methods. Table II lists the respiratory data obtained following intravenous injection of 2 gm. of sodium salicylate. Immediate respiratory stimulation was observed in each instance with development of a slight respiratory alkalosis. A group mean increase in oxygen consumption of 18 per cent was noted. Our interpretation of these effects is the same as for similar experiments previously cited for the dog. Also listed in Table II are the respiratory data obtained in these same five subjects as they ingested 8 gm. of acetylsalicylic acid (enteric-coated) per day for a period of three days. Continued hyperventilation is in evidence.

TABLE II

RESPIRATORY DATA IN NORMAL MAN

Period	Subject	Plasma Salicylate (mg. %)	Oxygen Consumption (ml./min.)	Expired Volume, L./min. (BTPS)			Alveolar CO_2 Tension, mm. Hg			Respiratory Frequency, Breaths/min.		
				Room Air	3% CO_2	5% CO_2	Room Air	3% CO_2	5% CO_2	Room Air	3% CO_2	5% CO_2
Control	RM (5)*	0	275	7.59	16.25	27.7	41	43 2	47	7.9	13.5	16
	AM (3)	0	22.	4.28	9.7	12.41	41.3	43 7	48.7	5	8.2	9.7
	BS (4)	0	273	8.1	16.13	27.9	39	41 7	44.3	8.1	10	13.4
	MC (3)	0	203	4.97	7.63	10.58	44.4	46 3	51.8	8	9.4	10.4
	MT (3)	0	224	6.7	13.4	19.3	38.7	42 3	44.7	8.3	11.2	13.4
	Mean	..	239	6.33	12.62	19.57	40.9	43 4	47.3	7.5	10.5	12.6
Twenty-five minutes after 2 gm. Na salicylate intravenously	RM (2)	22	275	8.62	19.25	32.2	39	42	49	9.0	17.2	18
	AM (2)	22	277	5.56	11.11	14.75	37.6	.. .	45.4	5.2	6.4	7.8
	BS (2)	22	295	7.68	19.6	34.6	37.3	37 3	40.7	7.6	12.8	17.8
	MC 2)	27	233	5.95	7.65	11.32	44.5	48.6	52.0	7.8	9.2	10.8
	MT (2)	27	230	8.55	13.05	23.0	35.0	36.5	43.2	8.6	12.6	16.4
	Mean	24	264	7.27	14.14	23.2	38.7	41.1	46.0	7.6	11.6	14.2
Oral aspirin, 2nd day, 3 gm./day	RM	21	...	7.9	18.2	24.1	38.4	43.7	44	11.4	15.2	19
	AM	32	...	5.71	12.85	17.6	36.4	41.4	46.2	5.0	7.6	8.6
	BS	29	...	9.1	27.2	36.6	37.6	37.3	40.4	9.6	21.7	22.6
	MC	35	...	6.94	10.7	11.82	39.5	43.2	49.2	8.6	11.6	14
	MT	37	...	7.68	15	27.3	37.8	36.2	43.6	9.6	13	17.4
	Mean	31	...	7.47	16.79	23.5	37.9	40.3	44.7	8.8	13.8	16.3
24 hrs. after oral drug discontinued	RM	23	...	10	22.8	33.7	38.4	42.5	42.5	14.4	13.9	16
	AM	23	...	5.44	11.2	15.65	37	41.2	42.8	4.4	7.4	10.8
	BS	16	...	9.15	20.8	31.1	39.1	41.1	42.2	7.6	10.4	12.4
	MC	38	...	6.84	11.1	18.5	34	41.2	45.3	9.2	11	13.8
	MT	35	...	7.68	20	30.3	33.6	36.2	38.8	11	15.8	18
	Mean	27	...	7.82	17.2	25.8	36.2	40.4	42.3	9.3	11.7	14.2

* Figures in parentheses refer to number of determinations made on each subject.

The mean ventilatory response to carbon dioxide in these subjects is indicated in Figure 7. On this graph the respiratory stimulus is considered as the alveolar CO_2 tension and is plotted on the abscissa. The response, plotted on the ordinate, is the alveolar ventilation ratio (VaR). The calculated alveolar ventilation while the subject is breathing room air during the control period is defined as the alveolar ventilation ratio equal to 1. All other alveolar ventilation ratios are calculated in reference to this value. Thus an alveolar ventilation value which is twice the control alveolar ventilation while breathing room air is expressed as an alveolar ventilation ratio of 2.

The immediate effect of sodium salicylate is to shift the stimulus-response curve to the left but it remains parallel to the control curve. The prolonged action following oral administration of the drug results not only in a further shift to the left but also in a steeper ascent. Since the slope of such a curve $\left(\frac{\Delta VaR}{\Delta P_aCO_2}\right)$ is a quantitative estimate of the ventilation response for any increment in alveolar CO_2 tension (stimulus), it may be regarded as an index of the "sensitivity" of the respiratory center to carbon dioxide. The response of the body to prolonged ingestion of acetylsalicylic acid is to cause a 24 per cent increase in sensitivity of the respiratory center to carbon dioxide (slope constant of ventilation line changes from 0.33 to 0.41), as well as maintained hyperventilation. The absence of change in sensitivity with rapid intravenous administration of salicylate and the further increase in sensitivity on the first day after cessation of the

orally administered drug, in the face of a slight fall in mean blood salicylate level, suggest that this change is not due directly to the salicylate but is the result of secondary changes in blood and tissue attending prolonged hyperventilation. Similar respiratory data have been obtained in

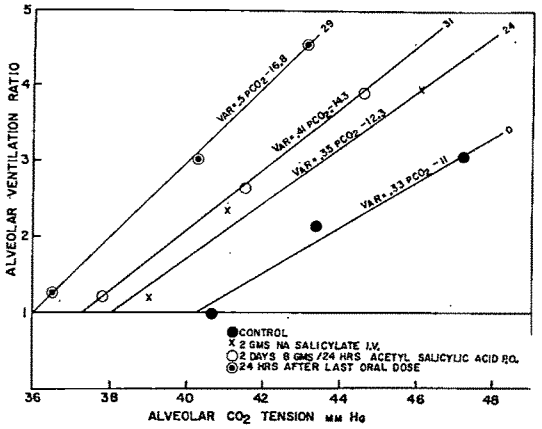


FIG. 7. Mean ventilatory stimulus-response curves to CO_2 in normal man; equations noted are calculated by method of least mean squares and define the lines fitted to mean value points. Data are from Table II or calculated from it (VaR); values at end of line are mean blood salicylate levels for each curve.

three patients receiving prolonged salicylate therapy. The mean alveolar CO_2 tension in this group was 25 mm. Hg and oxygen consumption was increased by 30 per cent. No additional information was added other than to establish that the maintenance of hyperventilation and increase in oxygen consumption is manifest in the presence of disease as well as in health.

Graphic Synthesis of Combined Respiratory Action (Direct and Metabolic). For convenience in quantitating individually the combined effects of salicylate which occur simultaneously in intact animal and man the graph of Figure 8 has been constructed. The simple mathematical relationships of alveolar CO_2 tension, alveolar ventilation and metabolic CO_2 production in the body are portrayed on the major area. Relative values are used, insofar as possible, in order to compare heterogeneous populations as well as to emphasize the generality of these relationships. The abscissa is the tension of CO_2 in the alveolar air. The normal value is arbitrarily selected at 40 mm. Hg. The ordinate represents relative CO_2 production. Whatever the absolute control value, it is represented by 1 on this axis. A similar ordinate is drawn for oxygen consumption but remains valid throughout any experimental procedure only so long as the respiratory gas exchange ratio is the same for any two time periods being compared. If the gas exchange ratio is 0.8 and the absolute value of CO_2 production at the control period is 80 cc. per unit of time, then it is clear that the similar

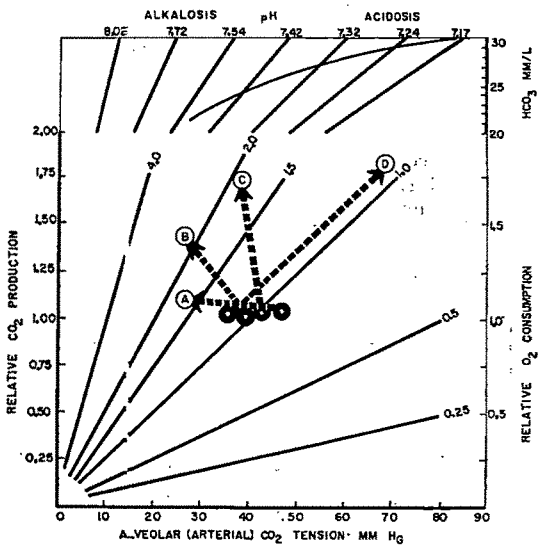


FIG. 8. Graphic representation of interrelation of alveolar CO_2 tension, metabolic CO_2 production and alveolar ventilation (portrayed as family of alveolar ventilation ratio isopleths radiating from origin); solid dots represent mean control alveolar points, and arrows indicate direction of movement of alveolar points in appropriate experiments to arrive at final values indicated by open circles. (See text for interpretation.) A, mean final alveolar point of dogs with pure respiratory stimulation following application of sodium salicylate to fourth ventricle of brain; B, final mean point for normal man receiving prolonged oral salicylate therapy with mean blood level of about 30 mg. per 100 cc.; C, mean final point for anesthetized dogs receiving 100 mg./kg. sodium salicylate intravenously; D, mean final point for curarized dogs receiving 100 mg./kg. sodium salicylate and maintained at constant ventilation in whole body respirator.

point on the oxygen consumption scale, indicated at relative value 1, represents an absolute oxygen consumption value of 100 cc. The only advantage in adding the oxygen consumption ordinate is that thinking is often in terms of this parameter rather than of CO_2 production. Since alveolar CO_2 tension is directly proportional to the level of metabolic CO_2 production, but inversely proportional to the magnitude of alveolar ventilation, alveolar ventilation isopleths may be represented as a family of straight lines radiating from the origin of this plot. Alveolar ventilation ratios are indicated on the

appropriate lines. As the isopleths approach the vertical position the alveolar ventilation ratios converge rapidly to infinity since it is only at infinite ventilation that the alveolar CO_2 tension can remain zero. The normal alveolar point is at 40 mm. Hg alveolar CO_2 tension, relative CO_2 production = 1, and lies on the alveolar ventilation ratio line labelled 1. Pure vertical displacement of the alveolar point represents ventilatory response which is entirely secondary to changes in the level of CO_2 production. Since in this instance the tension of CO_2 in the alveolar air (and arterial blood) remains unaltered, the total respiratory economy of the body is static. On the other hand, ventilatory response of purely nervous origin results in a horizontal displacement of the alveolar point with resultant changes of CO_2 tension and hence in acid-base balance of the body. Combined effects are seen as vectoral resultants of the two "pure" cases cited. All possible variations in salicylate response must fall within the area defined by the extreme vectors determined experimentally (points A and D). Point A represents the final alveolar value from pure central stimulant effect achieved in the dog by topical application of salicylate to the medulla. Point D, on the other hand, is the final alveolar point for a dog whose respiration is maintained at a constant value in a whole body respirator and can exhibit only the effects of salicylate-induced increase in oxygen consumption and carbon dioxide output. The alveolar point can move only up and to the right along the control alveolar ventilation isopleth. It is clear that if the ventilatory response does not keep pace with the increase in CO_2 production, respiratory acidosis develops. The usual effect seen in the lightly anesthetized animal or in man is a combination of the two with a dominant central stimulant action resulting in respiratory alkalosis (points B and C).

TABLE III

RESPIRATORY DATA IN DOGS BEFORE AND AFTER INTRAVENOUS INJECTION OF 100 MG./KG. SODIUM SALICYLATE

Dog	Respiratory Frequency (breaths/min.)	Minute Volume (L./min.)	O_2 Consumption (ml./min.)	P_aO_2 (mm. Hg)	P_aCO_2 (mm. Hg)	Blood pH	Arterial O_2 Content (vol. %)	Arterial CO_2 Content (vol. %)	Venous O_2 Content (vol. %)	Venous CO_2 Content (vol. %)
Control										
1	9.5	1643	16	96	44.6	7.50	15.9	48.8	11.7	52.4
2	6	1770	14	93	45.7	7.42	13.2	53.3	8.9	55.3
3	5	834	6	100	37.4	7.44	14.0	48.6	11.5	49.9
4	10	1430	8	99.4	41.5	7.41	14.2	45.7	11.3	47.7
5	10	2000	12	99	46.6	7.42	17.1	46.0	13.5	48.6
6	8.5	2978	17	94	45.1	7.47	17.5	49.5	14.3	50.1
7	7	1890	12	104	36	7.41	18.1	44	14.5	47.7
8	24	3504	12	108	35	7.44	12.9	46.5	9.2	....
Mean	10	2006	12	99.1	41.5	7.44	15.36	47.8	11.8	50.2
25 Minutes after Salicylate										
1	15	3960	32	101	41.7	7.53	15.9	47.2	9.3	52.7
2	5.5	1975	24	.....	46.4	7.47	13.0	48.7	7.4	53.3
3	6.5	1625	10	100	40.5	7.40	14.3	47.7	10.0	51.9
4	13	2600	14	102	40.3	7.45	13.5	44.8	9.8	48.9
5	14	3206	18	95	36.6	7.52	18.6	38.8	13.2	42.3
6	10	4550	32	94	44.4	7.47	17.3	48	12.5	55.2
7	9.5	2907	22	103	37	7.50	15.4	43.1	8.9	50.4
8	27	5606	19	126	20	7.47	15.1	38.1	9.7	44.7
Mean	12.5	3303	21	216	38.3	7.48	15.36	44.6	10.1	49.9

The simultaneous events which take place in the blood during any acute experiment are represented at the top of Figure 8. A range of blood bicarbonate values is indicated in the upper right section. The common alveolar

(arterial) CO_2 tension abscissa allows one to draw a CO_2 dissociation curve which is indicated as the curved line in the figure. pH isopleths are calculated from the Henderson-Hasselbalch equation and are represented by the family of straight lines in this portion of the figure. The consumption caused by salicylate is met by widening the arteriovenous oxygen difference but the other half is met only at the expense of increasing cardiac output. This is effected largely by an increase in stroke volume since the pulse rate increases only slightly.

TABLE IV

CIRCULATORY DATA IN DOGS BEFORE AND AFTER INTRAVENOUS INJECTION OF 100 MG./KG. SODIUM SALICYLATE

Dog	Pulse Rate (beats/min.)	Carotid Pressure (S/D mm. Hg)	Right Ventricular Pressure (S/D mm. Hg)	A-V O_2 diff.	Cardiac Output (L./min.)	Stroke Volume (ml.)
			Control			
1	150	165/126	29.5/1	4.2	3.9	26
2	125	162/120	13/0	4.3	3.45	27.5
3	160	165/125	24/0	2.5	2.48	15.5
4	78	180/138	29.5/0	2.9	2.93	37.5
5	81	167/125	24/0	3.6	3.47	43
6	150	225/175	37/0	3.2	5.60	37.3
7	170	155/110	30/0	3.6	3.55	21
8	145	134/100	31/0	3.7	3.32	23
Mean	133	169/127	27.9/0	3.5	3.59	28.8
			25 Minutes after Salicylate			
1	174	165/130	35/1	6.6	5.00	28.7
2	126	164/128	2_/0	5.6	4.32	34.3
3	120	155/120	25/11	4.3	2.40	20
4	78	179/143	29/1	3.7	3.90	50
5	78	174/137	27/0	5.4	3.34	43
6	150	218/175	34/0	4.8	6.66	44
7	182	147/112	3_/1	6.5	3.40	18.7
8	180	172/120	42/0	5.4	3.56	19.8
Mean	136	172/133	3_/2	5.3	4.07	32.3

arterial point is determined by projecting vertically from the alveolar point until the CO_2 dissociation curve is met. The pH is then read directly. This method is not applicable to chronic experiments in which there may be compensatory excretion of bicarbonate unless one has sufficient data to draw the new dissociation curve.

Circulatory Adjustments. It is of major interest to know how the body adjusts, in respect to the cardiovascular system, to the increase in oxygen consumption caused by salicylate. Tables III and IV list the data obtained to help answer this question. The low arterial oxygen content noted during the control period, and the wide alveolo-arterial oxygen gradient, are characteristic of dogs under pentobarbital anesthesia.[15] It is noted that about half of the increase in oxygen

Pressures in both the systemic and lesser circuit increased promptly following intravenous administration of sodium salicylate. Figure 9 illustrates one such experiment. Within fifteen minutes systemic pressure tended to return to the approximate control value, although in one instance it fell below and in one instance (illustrated) it remained above the control level for one hour. The gradual fall in systemic pressure to normal in the presence of an elevated cardiac output suggests peripheral vasodilatation and fall in vascular resistance to flow. This probably is related to the mechanism by which salicylates act to lower body temperature. Right ventricular pressure remained elevated throughout the course of all experiments; the extent of rise could be wholly accounted for on the basis of the increase in pulmonary blood flow.

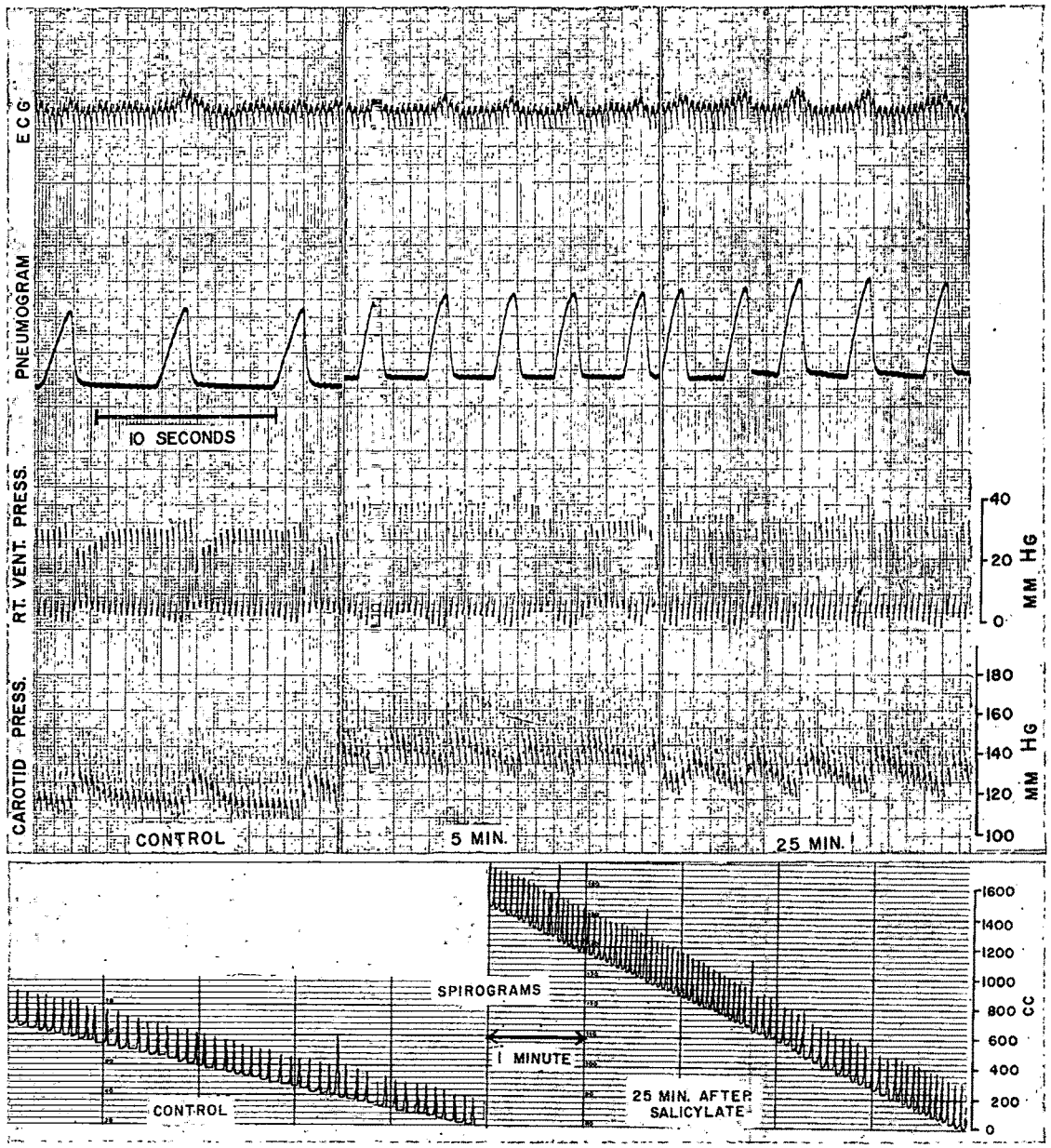


FIG. 9. Representative records o dogs after intravenous injection of 100 mg./kg. sodium salicylate.

The initial pressure response is independent of the release of epinephrine since it occurs under adrenergic blockade (dibenzylene). It is probably independent of nervous influence altogether since the initial response is also seen in the presence of complete ganglionic blockade (tetraethyl ammonium chloride). This is interpreted to mean that salicylate probably has a direct stimulating action on cardiac muscle. In the experiments with tetraethyl ammonium chloride the initial rise in systemic pressure following salicylate was followed much more promptly by a return to the control level than in animals not under the influence of tetraethyl ammonium chloride.

No change in the electrocardiogram was observed in these experiments.

A comprehensive summary of the respiratory

and circulatory changes listed in or derived from Tables III and IV is portrayed in Figure 10.

COMMENTS

The early clinical reports which indicated a metabolic stimulating effect of salicylate made little comment on this response.[9] More recent studies have raised the interesting speculation as to whether the therapeutic actions of salicylates may in some way be related to the rise in tissue oxygen consumption.[10,13] Little attention has been given to the major effect that the change in respiratory gas metabolism may have on the interpretation of both the respiratory response and the ultimate change in acid-base balance. There is no clue to the biochemical mechanism by which this stimulation is mediated, although the similarity of the dinitrophenol effect has been pointed out.[13]

The change in sensitivity of the respiratory center is not surprising since this altered state is readily observed in other situations of chronic hyperventilation, whether it is caused by prolonged hypoxia[16] or mechanical hyperventilation.[17] The order of change coincides well with general predictions concerning alterations in CO_2 and bicarbonate discussed elsewhere.[18]

The present series of experiments confirms the more recent view that in adult man respiratory alkalosis is the anticipated alteration in acid-base balance associated with the effects of salicylate.[8] The older studies purporting to show a metabolic acidosis are almost certainly the result of faulty interpretation of blood CO_2 combining power values. The fatal effects of salicylate during the terminal period require further clarification, but the older studies demonstrating a late fall in blood pH in the experimental animal may very likely be related to an acidosis which is not metabolic in the sense in which this term is ordinarily employed. If for any reason the ventilatory mechanism fails to keep pace with the increase in CO_2 production secondary to salicylate stimulation of metabolism, an acidosis results. It is, however, a respiratory acidosis since it must invariably be associated with a rise in alveolar (and arterial) CO_2 tension without a fall in plasma bicarbonate.

The circulatory effect raises the interesting question as to whether, with large doses of salicylate, cardiac output also is increased. This would be of particular importance in such states as rheumatic carditis. A direct action upon the heart has been sought in isolated tissue work in the past but no consistent changes have been noted. High concentrations may be directly depressant, but lower concentrations have a stimulating effect on rate and amplitude of contraction.[19,20] A rise in systemic blood pressure has been noted in the past even in the face of vasodilatation.[21,22]

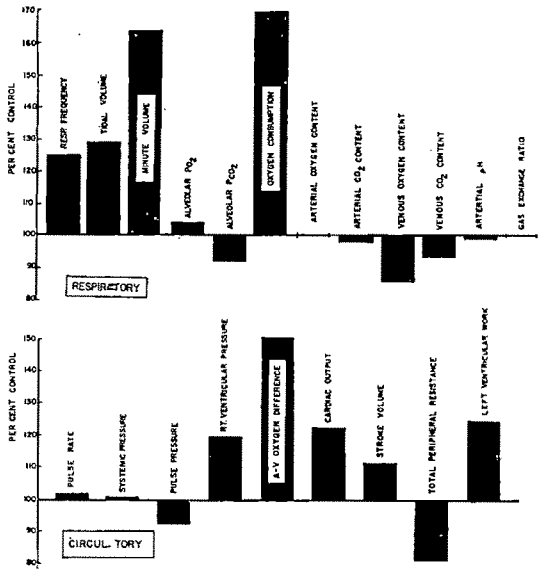


FIG. 10. Mean changes in respiratory and circulatory measurements plotted as per cent of control value, following intravenous injection of 100 mg./kg. sodium salicylate. Estimates calculated from data of Tables III and IV.

SUMMARY

1. Sodium salicylate has a direct respiratory stimulant action. The locus of this effect is in the medulla and is independent of the aortic and carotid chemoreceptor areas.

2. Sodium salicylate also is a profound metabolic stimulant and may increase oxygen consumption two-fold. A dosage-response relationship has been established in dogs, the maximum effect being noted at dosages of about 100 mg./kg. when the drug is given intravenously. The source of metabolic increase is primarily skeletal muscle and is independent of the central and peripheral nervous system. It is also not dependent on epinephrine release.

3. In normal man prolonged salicylate administration increases the "sensitivity" of the respiratory center to carbon dioxide.

4. The principal change in acid-base balance in the intact dog and adult human is the development of respiratory alkalosis. When ventilation is inadequate, respiratory acidosis may

develop. A diagram is presented to illustrate and differentiate the combined actions of salicylate on the respiratory mechanism—direct stimulation and secondary stimulation via increase in level of metabolism.

5. The circulatory system meets the increased level of oxygen consumption by widening the auriculoventricular oxygen difference and increasing cardiac output. There is an initial rise in systemic pressure, which usually returns to normal within fifteen minutes. Right ventricular pressure rises early and remains high for at least one hour. The magnitude of rise is directly proportional to the increase in cardiac output. The initial pressure rise is not caused by epinephrine release and can also be demonstrated in the presence of ganglionic blockage (tetraethyl ammonium chloride). It is concluded that this is a direct cardiac muscle effect of salicylate.

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Effect of Salicylate on the Acid-Base Equilibrium of Patients with Chronic CO_2 Retention Due to Pulmonary Emphysema*

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A FEW years ago it was demonstrated that salicylate may induce respiratory alkalosis in animals and humans[1–3] and recently it was shown that salicylate increases hyperventilation induced by CO_2 inhalation in normal human subjects.[4] Therefore it seemed logical to investigate the effects of salicylate in patients with chronic retention of CO_2 due to pulmonary emphysema.

METHODS

Patients with chronic CO_2 retention due to pulmonary emphysema without evidence of acute or recent respiratory infection were studied under basal conditions.

A first series of observations was designed to study acute effects of sodium salicylate on the acid-base equilibrium of the blood of patients breathing room air. After a control period, 6 to 8 gm. of sodium salicylate dissolved in 250 to 300 cc. of isotonic sodium chloride solution were administered intravenously over a period of forty-seven to sixty-seven minutes. Samples of arterial blood were collected anaerobically from the brachial artery through an indwelling needle at appropriate intervals during the control period and after the administration of salicylate. Heparin was used as anticoagulant, and blood samples were analyzed immediately. Whole blood pH was measured with a Beckmann model G pH-meter at room temperature and corrected to body temperature value.[5] Arterial plasma CO_2 content and arterial blood oxygen content and capacity were determined by the methods of Van Slyke and Neill.[6,7] Plasma CO_2 tension was calculated from the Henderson-Hasselbalch equation. The plasma salicylate level was determined by the method of Coburn.[8]

In a second series of experiments the patients breathed room air during the control period. After the control period they breathed pure oxygen administered by mask for thirty-five to ninety minutes; then the acute effects of the intravenous administration, over a forty to sixty-five minute period, of 6 to 8 gm. of sodium salicylate dissolved in 300 to 500 cc. of isotonic sodium chloride solution were observed while the patients continued to inhale pure oxygen. Samples of blood were collected, as in the first series of experiments, during the control period and during the period of oxygen inhalation before and after administration of salicylate. Blood pH, plasma CO_2 content, blood oxygen content and capacity and plasma salicylate were determined as in the first series of experiments.

The third series of observations dealt with the effects of acetylsalicylic acid administered orally over a period ranging from three to twenty-two days. After a control period patients were given approximately 0.6 to 1.8 gm. of aspirin every six hours, day and night. Blood samples were collected anaerobically at the same time of day, the patients being in a basal state. In some experiments arterial blood was collected and analyzed as in the first two series of experiments; in other experiments only venous blood drawn from the antecubital vein without stasis was analyzed; in some experiments both

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venous blood and an occasional arterial blood sample were analyzed. Blood samples were always drawn just before the administration of a dose of aspirin.

RESULTS

The results observed are summarized in Tables I, II and III.

A. Acute effects of intravenous administration of sodium salicylate. As can be seen in Table I, five patients received 6 to 8 gm. of sodium salicylate intravenously within forty-seven to sixty-seven minutes. The maximal plasma salicylate levels noted occurred three to ninety-two minutes after termination of the salicylate injection and ranged from 21.1 to 51.9 mg. per

TABLE I

EFFECT OF INTRAVENOUS ADMINISTRATION OF SODIUM SALICYLATE ON ARTERIAL BLOOD pH, pCO_2, BICARBONATE AND OXYGEN SATURATION IN PATIENTS WITH ANOXEMIA AND HYPERCAPNIA DUE TO CHRONIC PULMONARY EMPHYSEMA

Observation Number	Patient, Age	Time (min.)	Arterial Blood pH	Arterial Plasma pCO_2 (mm. Hg)	Arterial Plasma HCO_3^- (mEq./L.)	Plasma Salicylate (mg. %)	Arterial Blood Oxygen Saturation (%)
1	H. W., 64	− 57	7.33	82	42.0	0	69.7
		− 5	7.32	81	40.7	0	.....
		0 to +60	Infusion of 6 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution				
		+ 63	7.35	78	41.7	21.1	76.8
		+123	7.35	73	38.9	20.8	80.2
		+188	7.38	64	36.7	....	91.8
		+263	7.35	72	38.5	14.8	71.8
2	F. B., 63	− 25	7.34	68	35.7	0	79.5
		− 5	7.33	69	35.4	....	79.5
		0 to +47	Infusion of 6 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution				
		+ 79	7.39	58	34.2	25.0	85.0
		+139	7.40	56	33.9	26.5	83.0
		+195	7.43	53	34.1	21.1	80.0
3	J. H., 63	− 30	7.34	59	31.0	....	.....
		− 5	7.32	62	30.9	0	80.4
		0 to +60	Infusion of 8 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution				
		+ 80	7.33	60	30.6	26.6	90.4
		+135	7.36	54	29.7	25.0	.....
		+195	7.42	46	29.1	23.5	93.6
		+220	7.43	44	28.5	22.2	90.4
4	C. F., 64	− 15	7.33	60	30.4	0	77.8
		− 5	7.33	61	31.2	0	75.8
		0 to +67	Infusion of 8 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution				
		+ 82	7.41	49	30.3	51.9	83.1
		+130	7.43	46	29.8	47.2	86.2
		+175	7.38	51	29.3	44.7	77.4
		+210	7.38	50	28.4	41.6	75.0
5	X. A., 62	− 13	7.27	73	32.7	0	17.8*
		− 8	7.29	69	32.0	0	18.2*
		0 to +60	Infusion of 8 gm. of sodium salicylate dissolved in 250 cc. of isotonic sodium chloride solution				
		+ 80	7.37	55	30.8	23.4	18.5*
		+135	7.41	49	30.0	24.5	19.2*
		+185	7.43	45	29.1	23.5	19.8*

* Arterial blood oxygen content (vol. %).

cent. The administration of salicylate resulted in a decrease of plasma pCO_2, the maximal decrease being reached from sixty-three to one hundred and sixty minutes after the end of the infusion. The plasma pCO_2 was still markedly depressed when the observations were discontinued, as long as 203 minutes after cessation of the salicylate administration. The arterial blood pH rose, the maximal rise coinciding with the maximal decrease in the plasma pCO_2. The plasma bicarbonate decreased. The oxygen content and saturation of the arterial blood rose in four of the five patients; in the remaining patient, in whom only the oxygen content of the arterial blood was measured, the oxygen content rose as in the other four patients.

B. Acute effects of the intravenous administration of sodium salicylate during inhalation of pure oxygen. As can be seen in Table II, five experiments were performed on four patients. In all five observations administration of pure oxygen by mask resulted in a rise in plasma pCO_2 as well as in oxygen content and saturation of the arterial blood and decrease in plasma pH. The plasma bicarbonate rose slightly but consistently. With inhalation of pure oxygen continued, 6 to 8 gm. of sodium salicylate dissolved in 300 to 500 cc. of isotonic sodium chloride solution were administered intravenously. The plasma salicylate level reached a maximum ranging from 20.0 to 26.3 mg. per cent. In all five experiments (except in the first of the two observations made on patient F. B., Table II) administration of sodium salicylate resulted in decrease of the plasma pCO_2 towards, to or below its level before oxygen inhalation was begun, and a rise of the blood pH towards or above its value before initiation of oxygen inhalation. The maximal effect was reached 20 to 125 minutes after the end of the salicylate infusion but some effect persisted 115 to 125 minutes after the end of the infusion. The oxygen content and saturation of the arterial blood remained essentially unchanged by administration of salicylate. In the first observation made on patient F. B. (Observation 2 of Table II), in which the salicylate blood level was the lowest in this series of experiments, the plasma pCO_2 and pH did not change after the salicylate injection. However, it is probable that even at such a low salicylate level a further rise in pCO_2 and decrease in pH during the continued inhalation of oxygen was obviated as is indicated by the fact that, as the salicylate level began to decrease, the pCO_2 rose further and the pH fell. Furthermore, as can be seen in Table II, the same patient, F. B., responded to the salicylate injection as did the other patients when, during a second experiment (Observation 3 of Table II) performed fourteen days after the first experiment, a higher blood salicylate level was obtained by administration of 8 gm. instead of 6 gm. of sodium salicylate.

C. Effects of continued administration of aspirin by mouth. The results observed are summarized in Table III. Four patients, H. W., F. B., B. L. and C. F., reacted in a similar manner. In Observation 1 patient H. W. was given 1.5 gm. of aspirin every six hours for six days. On the sixth day of aspirin administration the plasma salicylate level was 16 mg. per cent. In Observation 2 patient F. B. was given 2 gm. of aspirin every six hours for one day, than 2 gm. every eight hours for two days. In Observation 3 patient B. L. received 1.8 gm. of aspirin every eight hours for nine days, and the plasma salicylate level ranged between 16.5 and 22.6 mg. per cent. In Observation 4 patient C. F. was given 0.9 gm. of aspirin every eight hours for five days, then 0.6 gm. every six hours for four days, and the plasma salicylate level ranged between 22.8 and 30.7 mg. per cent.

In the first two observations the administration of aspirin resulted in a decrease of arterial plasma pCO_2 and bicarbonate and a rise in the arterial blood pH. In patients B. L. and C. F. the total CO_2 of the venous plasma fell progressively. When administration of aspirin was discontinued after six, three and nine days, respectively, in patients H. W., F. B. and C. F., arterial plasma pCO_2 and bicarbonate, as well as the arterial blood pH, or the venous plasma CO_2 returned toward, to or even beyond control levels. In Observation 5 patient L. B. received 1.8 to 2.4 gm. of aspirin every eight hours and the plasma salicylate levels ranged between 21 and 40.3 mg. per cent. In this patient, only the higher concentrations of salicylate in the blood were associated with a significant fall in the total CO_2 of the venous plasma. Finally, in Observation 6 patient H. W., who had responded previously to oral administration of 6 gm. of aspirin per day (Observation 1), did not respond at all to the administration of a dose of aspirin slightly larger than that given in Observation 1. Whether or not this lack of response was related to the upper respiratory infection which the patient was noted to have on the seventh day of aspirin administration cannot be determined.

TABLE II

EFFECT OF INTRAVENOUS SODIUM SALICYLATE DURING INHALATION OF 100% OXYGEN ON ARTERIAL BLOOD pH, pCO_2, BICARBONATE AND OXYGEN SATURATION IN PATIENTS WITH ANOXEMIA AND HYPERCAPNIA DUE TO CHRONIC PULMONARY EMPHYSEMA

Observation Number	Patient, Age	Time (min.)	Arterial Blood pH	Arterial Plasma pCO_2 (mm. Hg)	Arterial Plasma HCO_3^- (mEq./L.)	Plasma Salicylate (mg. %)	Arterial Blood Oxygen Saturation (%)
1	H. W., 64	−114	7.39	69	40.6	.....	79.2
		− 94	7.39	68	39.8	....	77.4
		− 90 Beginning of inhalation of 100% oxygen by mask					
		− 35	7.28	92	41.7	....	100
		− 4	7.26	96	41.7	0	97.5
		0 to +65 Infusion of 6 gm. of sodium salicylate dissolved in 500 cc. of isotonic sodium chloride solution					
		+ 95	7.31	86	42.1	26.3	100
		+140	7.32	82	41.1	25.0	100
		+190	7.34	76	39.7	23.5	100
2	F. B., 63	− 95	7.38	59	33.7	0	80.2
		− 75	7.39	58	34.2	....	75.2
		− 65 Beginning of inhalation of 100% oxygen by mask					
		− 30	7.32	72	35.8	0	100
		− 2	7.31	75	36.5	0	100
		0 to +40 Infusion of 6 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution					
		+ 68	7.31	74	36.1	20.0	100
		+125	7.31	75	36.5	19.0	98.8
		+182	7.31	75	36.4	....	100
		+213	7.28	78	35.3	18.7	84.9
3	F. B., 63	− 60	7.35	54	29.2	....	90.4
		− 50	7.37	52	29.3	....	85.8
		− 45 Beginning of inhalation of 100% oxygen by mask					
		− 30	7.25	72	30.4	....	98.7
		− 4	7.24	76	31.6	0	100
		0 to +45 Infusion of 8 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution					
		+ 75	7.34	60	31.2	25.0	100
		+120	7.31	63	30.9	23.5	.....
		+160	7.28	69	31.5	23.0	100
4	L. B., 61	− 45	7.35	63	33.5	....	85.0
		− 40	7.36	61	33.6	....	87.6
		− 35 Beginning of inhalation of 100% oxygen by mask					
		− 16	7.28	76	34.8	....	.....
		− 5	7.28	77	35.3	0	99.6
		0 to +50 Infusion of 8 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution					
		+ 70	7.37	63	35.2	25.0	100
		+125	7.36	65	35.2	24.5	.....
		+174	7.34	65	34.4	24.4	94.6
5	D. F., 61	− 69	7.38	54	31.6	....	92.5
		− 64	7.38	54	31.7	....	89.4
		− 60 Beginning of inhalation of 100% oxygen by mask					
		− 34	7.33	64	32.8	....	.....
		− 10	7.32	65	32.4	0	100
		0 to +50 Infusion of 8 gm. of sodium salicylate dissolved in 300 cc. of isotonic sodium chloride solution					
		+ 65	7.37	54	30.4	24.2	100
		+115	7.39	51	29.8	24.0	.....
		+165	7.38	52	29.8	22.3	.....

TABLE III

EFFECT OF ORAL ADMINISTRATION OF ASPIRIN ON ARTERIAL BLOOD pH, pCO_2 AND BICARBONATE AND/OR TOTAL CO_2 CONTENT OF THE VENOUS BLOOD IN PATIENTS WITH ANOXEMIA AND HYPERCAPNIA DUE TO CHRONIC PULMONARY EMPHYSEMA

	Time (days)	Arterial Blood pH	Arterial Plasma pCO_2 (mm. Hg)	Arterial Plasma HCO_3^- (mEq./L.)	Plasma Salicylate (mg. %)	Venous Plasma Total CO_2 (mEq./L.)
Observation 1	− 4	7.28	67	30.8	....	....
Patient H. W. (64 yr.)	− 1	7.35	58	30.8	....	....
	0	Administration of aspirin started, 6 gm. orally daily				
	+ 3	7.40	44	26.3	....	....
	+ 5	7.40	40	24.3	16.0	....
	+ 6	Administration of aspirin stopped				
	+ 9	7.31	68	33.2	....	....
Observation 2	− 1	7.28	66	30.0	....	....
Patient F. B. (63 yr.)	0	Administration of aspirin started, 8 gm. orally daily				
	+ 1	Aspirin reduced to 6 gm. orally daily				
	+ 3	7.36	46	25.4	....	....
	+ 3	Administration of aspirin stopped				
	+ 6	7.32	56	27.8	....	....
Observation 3	− 1	....	..	....	....	31.2
Patient B. L. (59 yr.)	0	Administration of aspirin started, 5.4 gm. orally daily				
	+ 1	....	..	....	....	30.7
	+ 5	....	..	....	22.6	28.5
	+ 6	....	..	....	16.5	30.0
	+ 7	....	..	....	22.6	28.9
	+ 8	....	..	....	21.6	27.0
Observation 4	− 2	....	..	....	....	36.3
Patient C. F. (64 yr.)	0	....	..	....	....	37.4
	0	Administration of aspirin started, 2.7 gm. orally daily				
	+ 2	....	..	....	22.8	35.6
	+ 4	....	..	....	30.7	30.6
	+ 5	Aspirin reduced to 2.4 gm. orally daily				
	+ 6	....	..	....	24.2	31.9
	+ 9	Administration of aspirin stopped				
	+ 9	....	..	....	10.5	39.3
	+11	....	..	....	0	41.2
	+13	....	..	....	....	40.7
Observation 5	− 1	....	..	....	....	42.0
Patient L. B. (61 yr.)	0	....	..	....	....	43.8
	0	Administration of aspirin started, 7.2 gm. orally daily				
	+ 3	....	..	....	40.3	36.5
	+ 4	Aspirin reduced to 5.4 gm. orally daily				
	+ 5	....	..	....	34.0	40.5
	+ 7	....	..	....	28.6	40.8
	+10	....	..	....	21.0	43.1
	+11	Aspirin increased to 6.3 gm. orally daily				
	+13	....	..	....	26.8	41.7
Observation 6	− 2	7.38	57	32.6	0	....
Patient H. W. (64 yr.)	0	7.38	59	34.0	....	....
	0	Administration of aspirin started, 6.9 gm. orally daily				
	+ 4	7.38	53	30.5	20.0	....
	+ 7	7.36	60	33.0	20.0	....
	+11	7.39	54	31.9	20.0	....
	+15	7.37	55	30.9	19.7	....
	+22	7.31	76	37.0	19.0	....

In one patient not included in Table III oral administration of aspirin was begun six hours after completion of Observation 5 noted in Table II. This experiment, intended to study the effect of prolonged aspirin administration, could not be continued because of the development of an acute psychosis on the second day of oral administration of aspirin. At this time the plasma salicylate level was 41.8 mg. per cent. Administration of aspirin was discontinued and within twenty-four hours, at which time the plasma salicylate concentration was 26.2 mg. per cent, the psychosis cleared completely. Most patients became irritable during the acute and prolonged experiments involving salicylate administration. Two patients developed coarse tremor of the hands during the period of oral administration of aspirin.

COMMENTS

It is now well established that salicylate stimulates the respiratory center in animals and men.[1–4] However, the exact site of the stimulation is still not definitely known. Some authors[9] believe that salicylate stimulates the respiratory center both directly and via the sinoaortic nerves, whereas others[10] are of the opinion that stimulation of the respiratory center is only reflex in nature. Furthermore, the precise *modus operandi* of this stimulation is also unknown, although Alexander, Spalter and West[4] believe that at least one mechanism whereby salicylate acts on the respiratory center is that of sensitizing it to CO_2. Whatever the answer to these questions may be, it seems clear from our first series of experiments that, just as in normal animals or humans, salicylate can lower the arterial pCO_2 and raise the arterial pH in patients who retain CO_2 because of emphysema and because they seem to have become insensitive to CO_2. Although no direct measurements of pulmonary ventilation were made in the experiments described in this paper, hyperventilation was observed in the patients after intravenous administration of salicylate, and there is no doubt that the immediate effects of salicylate on the acid-base equilibrium are largely explained by the increase in pulmonary ventilation. It is of interest that salicylate lowers the arterial blood pCO_2 and raises the arterial blood pH, although by increasing the pulmonary ventilation it tends to correct the anoxemia which in such patients has become a potent respiratory drive.

A more direct demonstration of this property of salicylate was provided by the second series of experiments. It has been known for some time that in patients with emphysema who have become anoxemic and hypercapnic correction of the anoxemia by administration of oxygen intensifies hypercapnia and may result eventually in coma.[11,12] In the second series of experiments salicylate proved effective in reducing accumulation of CO_2 which had been induced by administration of oxygen. It would, of course, be of great interest to compare the efficacy of salicylate with that of the more conventional respiratory analeptics.

The third series of experiments demonstrated that oral administration of aspirin can reduce the retention of CO_2 and keep the blood pCO_2 depressed for as long as the administration of aspirin is continued, at least within the limits of duration of the present observations. However, only four of the six patients studied responded to a moderate dose of aspirin. One patient responded only to a rather large dose of the drug, and one patient who had reacted to a moderate dose of aspirin subsequently did not respond to a slightly larger dose.

Obviously study of a larger series of patients is necessary to permit a definite conclusion as to the efficacy of aspirin to lower and keep within the normal range the pCO_2 of patients with CO_2 retention due to chronic pulmonary emphysema. It must be remembered that collection of valid data on the prolonged administration of aspirin is difficult because the degree of CO_2 retention may fluctuate widely and rapidly with variations in the degree of bronchial obstruction, especially if a respiratory infection occurs. It should be pointed out that the doses of aspirin used caused irritability, occasionally coarse tremor of the hands and, on one occasion, temporary psychosis. Such side effects would seem to have been more marked in this group of patients than in a group of much younger patients who received similar or higher doses of aspirin for acute rheumatic fever.[13]

Whether the use of salicylate as a relatively long-acting respiratory stimulant will prove of therapeutic value remains to be determined by further work. The experience herein recorded suggests that it may be a useful adjunct in the treatment of patients with pulmonary emphysema in whom concurrence of severe anoxemia and hypercapnia makes administration of oxygen both a necessity and a hazard.

SUMMARY

In patients with chronic pulmonary emphysema which has led to anoxemia and hypercapnia it has been observed that: (1) The intravenous administration of 6 to 8 gm. of sodium salicylate over a period of approximately one hour lowers the arterial plasma pCO_2, raises the arterial blood pH and increases the arterial blood oxygen saturation. (2) When inhalation of oxygen has led to aggravation of hypercapnia intravenous administration of 6 to 8 gm. of sodium salicylate over a period of approximately one hour reduces the degree of hypercapnia (3) The oral administration of 3 to 7 gm. of aspirin per day in divided doses over a period of a few days gives variable results. In some patients relatively low plasma salicylate levels reduce the hypercapnia, in others only rather high levels are effective, in still others even such high levels are ineffective.

The therapeutic implications of these observations on the effect of salicylate in patients with chronic pulmonary emphysema, anoxemia and hypercapnia are pointed out but they must remain mere implications until more data are accumulated.

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Respiratory and Renal Effects of a Carbonic Anhydrase Inhibitor (Diamox) on Acid-Base Balance in Normal Man and in Patients with Respiratory Acidosis*

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INTEREST in the effect of carbonic anhydrase inhibition on the liberation of carbon dioxide from red blood cells in the pulmonary alveolar capillaries and on pulmonary ventilation has recently been stimulated by the introduction of diamox® (2 acetylamino-1-3,4 thiadiazole-5-sulfonamide), a potent inhibitor of this enzyme.[1] Roughton observed that sulfanilamide, a considerably less potent carbonic anhydrase inhibitor than diamox, interferes with the release of carbon dioxide in the lungs only during exhausting exercise.[2] This was also noted in recent studies with diamox.[3,4] It was also observed that sulfanilamide causes a consistent fall in the carbon dioxide content of serum.[5–7] The sequence of events leading to this has been debated. Beckman et al. believed that the drug brings about a primary renal loss of bicarbonate (metabolic acidosis) which quickly stimulates compensatory hyperventilation in an attempt to raise the level of arterial blood pH to normal.[8] Hartmann et al. reported that the primary event is ventilatory stimulation resulting in respiratory alkalosis and that subsequent increased renal excretion of bicarbonate was a compensatory attempt to adjust for the rise in arterial blood pH.[9] Since these studies with sulfanilamide were carried out the importance of carbonic anhydrase in the H^+—Na^+ ion exchange mechanism of the renal tubules which conserves fixed base has been demonstrated.[10]

The investigations of Nadell in normal subjects and in patients with respiratory acidosis confirmed the primary renal effects of diamox.[11] She noted a prompt fall in the level of plasma carbon dioxide content and arterial pH, followed by a delay of one to two weeks in the onset of a fall in arterial pCO_2, presumably as a result of respiratory stimulation, and a rise in arterial pH to normal values. This sequence of adjustments seemed unusual and stimulated our interest, particularly since Nadell did not carry out respiratory and gas exchange measurements.

It seemed essential to study the interrelationships of pulmonary ventilation, respiratory gas exchange, acid-base balance, blood electrolyte levels and renal electrolyte excretion rates in order to delineate the readjustments to the metabolic derangements induced by diamox. Such studies carried out in patients with chronic pulmonary emphysema and respiratory acidosis and in patients without lung, heart and kidney disease should contribute to an understanding of the action of the drug, help in the selection of patients who might benefit from its use and point out its limitations and possible contraindications.

The studies of Barach[12] suggested that intensification of acidosis following diamox ingestion due to a loss of plasma bicarbonate might predispose patients suffering from chronic

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emphysema associated with respiratory acidosis to develop carbon dioxide narcosis during oxygen inhalation. On the other hand, the studies of Comroe et al.[13] suggested that should a fall in arterial pCO_2 to 50 mm. Hg or less accompany a decrease in plasma bicarbonate in such patients, they might no longer exhibit a tendency to develop this complication. The variation in response to diamox noted among our patients suffering from chronic pulmonary emphysema associated with respiratory acidosis made it possible to evaluate both these concepts concerning the regulation of respiration.

TABLE I

MEASUREMENTS OF SOME ASPECTS OF PULMONARY FUNCTION

Patient	Age and Sex	Total Lung Capacity		Residual Volume / Total Lung Capacity (%)	Vital Capacity		Maximum Breathing Capacity		Oxygen Saturation of Arterial Blood (%)
		ml.	% Predicted		ml.	% predicted	L./min.	% predicted	
A. Patients with Chronic Pulmonary Emphysema									
M. A.	64, M	5326	104	57	3180	90	43	48	79
B. M.	53, F	4533	122	69	1710	66	26	42	94
H. R.	51, M	5651	100	70	1940	50	24	21	63
J. T.	68, M	....	...	..	1310	42	22	28	65
J. W.	63, M	3647	70	46	2800	78	28	30	62
B. Patients without Lung, Heart or Kidney Disease									
C. B.	47, M	6023	115	30	4535	112	191	166	96
H. B.	69, M	6017	120	40	3580	103	87	98	95
A. F.	59, M	5715	109	43	3465	95	100	99	95
J. N.	60, M	5437	110	53	2540	74	82	76	96
L. S.	78, M	4208	96	31	2885	95	85	107	94

METHODS

Studies were carried out in a group of five patients with chronic pulmonary emphysema associated with respiratory acidosis and in another group of five patients without lung, heart or kidney disease.

Three of the patients (J. T., J. W., H. R.) in the chronic emphysema group (Table I) exhibited electrocardiographic changes and x-ray findings compatible with cor pulmonale. Patient J. T. suffered from congestive heart failure, which was poorly controlled by a low-salt diet, digitalis, mercurial diuretics, ammonium chloride and aminophylline. Prior to hospitalization patient J. W. exhibited congestive heart failure, which was controlled by a low-salt diet and digitalis. Signs of congestive heart failure did not recur upon withdrawal of digitalis while in the hospital. Patient H. R., who had suffered from bronchial asthma since childhood, received only subcutaneous injections of adrenalin® when necessary for relief from acute asthmatic attacks for the duration of the study. Studies were carried out only when he had not experienced an asthmatic attack for several days. Patient B. M. had a complicating bronchiectasis. All except patient H. R. expectorated about 1 to 3 ounces of non-foul, mucoid sputum containing clumps of green and yellow matter. All patients studied ate the usual hospital diet except J. T. and J. W., who received a low-salt diet.

Studies were generally begun at about 8 A.M. with the patient in a basal state. To avoid possible variations in pulmonary ventilation due to posture, the head of the bed was raised to the same semi-reclining angle for each patient in every study. At the start of a study the patient voided and this urine was discarded. An indwelling arterial needle was then inserted into the lumen of the right brachial artery under local anesthesia with 2 per cent procaine

hydrochloride. With noseclip and mouthpiece in place, the patient inspired through one arm of a two-way valve and expired through another into a Tissot spirometer of 100 L. capacity. The dead space of the valve and mouthpiece was 55 ml. Tidal volume, respiratory rate and minute volume of respiration were recorded on a constant speed ink-recording drum attached to the spirometer.

Several two-minute respiratory periods were recorded over the next one-half to one hour. An arterial blood sample was then collected anaerobically, using heparin solution as an anticoagulant, during the middle of a seven-minute period of expired air collection. In the early phase of the studies only one arterial blood sample was drawn in a similar manner two to three hours after a dose of the drug. Later, the protocol was changed so that following the preliminary period of registration of respiration arterial blood was drawn just before the ingestion of a dose of diamox and two and four hours thereafter.

Urine was voided under oil immediately after each blood collection, followed by registration of the rectal temperature. In the intervals between blood collections, respirations were recorded and expired air collected during two minutes out of nearly every ten to fifteen minutes. Pulse rate and arterial blood pressure were also recorded at frequent intervals throughout each study.

Duplicate samples of expired air collected during the periods of blood withdrawal and a composite sample of air collected in the two-minute periods in the intervals between blood collections were analyzed for oxygen and carbon dioxide contents in a 0.5 ml. micro-Scholander gas analyzer.[14] The rate of oxygen consumed and carbon dioxide expired per minute and the respiratory exchange ratio (R.Q.) were calculated in the usual manner. All expired volumes in this and in the other ventilatory studies (Table I) were corrected to 37°C. saturated with water vapor and 760 mm. Hg (BTPS). Oxygen consumption and carbon dioxide output are expressed at 0° 760 mm. Hg (STPD).

Arterial blood oxygen and carbon dioxide contents[15] and arterial blood hemoglobin oxygen capacity[16] were analyzed in a Van Slyke manometric apparatus. Arterial pH was measured at room temperature with a sealed glass microelectrode in a model G Beckman pH meter and corrected to 37°C. using the factor of 0.014 per degree.[17] Arterial pCO_2 and plasma bicarbonate were calculated by the Henderson-Hasselbach equation. Plasma and urine sodium and potassium concentrations were analyzed in an internal standard flame photometer,[18] and urinary and plasma chloride concentrations by the method of Wilson and Ball.[19] Urine pH was measured with a sealed glass microelectrode in a Beckman model G pH meter and expressed at room temperature, 25°C. Urine carbon dioxide content was measured according to the method of Van Slyke and Neill.[15]

Residual air volume was measured by the method of Darling, Cournand and Richards[20] with modifications as previously published.[21] This, together with the vital capacity, measured the total lung volume. Vital capacity and maximum breathing capacity were measured in a Collins ventilometer. They represent the maximum volumes attained in a series of three observations at one sitting in a day. The patients had previously been trained in the performance of these tests. The results of these different measurements are summarized in Table I.

RESULTS

Immediate Effects of a Single Dose of Diamox in Patients with Pulmonary Emphysema and Elevated Arterial pCO_2 and in Patients without Lung, Heart or Kidney Disease. The major aspects of the results of studies carried out over four hours following a single dose of diamox in both groups of patients are summarized in Table II. All patients exhibited a prompt fall in plasma bicarbonate level and alkalinization of the urine associated with a diuresis of sodium and, to a lesser extent, of potassium bicarbonate, rarely with chloride ions. Data not included in Table II indicate that nearly all of the immediate electrolyte alterations may occur within one to two hours. The intensity of these responses varied considerably among the different patients. It was not related to the size of the dose of diamox, the initial level of plasma bicarbonate, arterial blood pCO_2, pH or oxygen saturation.

The level of arterial pCO_2 either remained unchanged, fell moderately (4 to 8 mm. Hg) or rose as much as 7 mm. Hg. (Table II.) Arterial pH either remained unchanged or fell as much as 0.07 units, reflecting the combined effects in each instance of the relative changes in arterial pCO_2 and plasma bicarbonate. The level of plasma chloride did not consistently rise as

TABLE II*

IMMEDIATE RESPONSE TO A SINGLE DOSE OF DIAMOX

Patient	Dose (mg./kg.)	Plasma		Arterial Blood				Urine										Respiration							
		$BHCO_3^-$ (mEq./L.)		pH_a		P_aCO_2		pH		$BHCO_3^-$ (mM/L.)		Na^+ (mEq./L.)		K^+ (mEq./L.)		Cl^- (mEq./L.)		$\dot{V}e/M^2$		R		$\dot{V}O_2/M^2$		$P_{A_{O_2}}$	
		Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.	Control	4 hr.
A. Patients with Chronic Pulmonary Emphysema																									
M. A.	4.2	29.7	27.1	7.30	7.29	63	59	6.79	7.45	8.06	.....	60	105	34	34	64	44	4.13	4.65	.80	.75	118	128	72	73
B. M.	6.3	26.3	22.4	7.32	7.28	53	49	5.66	7.65	0.06	15.55	85	168	29	82	104	53	5.98	7.28	.74	.76	166	199	83	89
H. R.	3.2	33.2	32.5	7.31	7.31	70	69	.....	.....	.....	.....	...	...	...	...	...	...	3.86	4.03	.75	.75	95	100	61	62
	3.5	34.4	31.6	7.28	7.23	76	79	6.23	7.37	14.99	32.73	138	325	60	94	161	145	4.10	5.45	.79	.81	90	82	57	54
	6.5	34.6	32.5	7.35	7.31	66	68	6.76	7.26	10.2	91.2	91	405	74	158	188	306	3.84	4.05	.74	.74	96	101	64	61
J. T.	4.2	34.2	32.3	7.27	7.25	79	79	5.96	7.26	1.0	110.4	13	143	21	79	19	36	7.62	8.21	.87	.87	199	216	60	60
J. W.	2.2	43.8	34.8	7.29	7.23	94	86	6.40	7.47	5.0	50.9	96	449	42	110	113	147	4.57	4.29	.83	.82	130	122	41	49
	4.4	36.2	30.1	7.26	7.21	82	79	5.56	7.38	0.07	76.2	49	140	30	63	80	61	3.95	3.87	.83	.83	117	115	54	54
B. Patients without Lung, Heart or Kidney Disease (Patient A. F. not studied.)																									
C. B.	3.8	21.2	19.1	7.38	7.31	37	39	6.37	7.48	2.4	20.3	197	332	103	144	...	125	4.20	5.27	.75	.83	123	138	102	103
H. B.	4.2	26.0	22.0	7.40	7.37	44	40	6.60	7.48	5.6	78.5	76	348	30	77	80	175	4.52	5.04	.84	.83	107	120	99	103
	4.8	24.7	20.8	7.42	7.39	39	36	6.79	7.51	12.9	104.9	65	249	31	52	50	67	5.25	4.67	.75	.75	101	96	97	101
J. N.	4.0	25.0	21.9	7.40	7.38	42	38	6.31	7.50	4.4	144.0	85	247	74	113	109	62	3.74	4.30	.78	.77	111	119	98	103
L. S.	3.7	23.4	21.8	7.43	7.33	37	44	6.24	7.37	.....	.....	31	124	18	45	36	48	3.33	3.31	.69	.70	96	92	97	89

* Symbols used in tables:
$\dot{V}e/M^2$ = minute volume of expired gas, i.e., ventilation, in L./min., BTPS
f = respiratory rate in breaths per minute
V_T = tidal volume, in ml. BTPS
$\dot{V}CO_2/M^2$ = CO_2 output, in ml./min./M^2 body surface, STPD
$\dot{V}O_2/M^2$ = O_2 consumption, in ml./min./M^2 body surface, STPD
R = the CO_2:O_2 exchange ratio or respiratory quotient
Sa = per cent oxygen saturation of arterial blood
pHa = pH of arterial blood
$PaCO_2$ = arterial pCO_2, in mm. Hg
$P_{A_{O_2}}$ = alveolar pO_2, in mm. Hg
rO_2 = inspired air oxygen content

plasma bicarbonate fell and there was no change in sodium and potassium plasma levels. The level of alveolar pO_2 rose moderately when arterial pCO_2 fell. These changes reflected improved alveolar ventilation and were accompanied by either a moderate degree of hyperventilation or an increase in the depth of breathing and a slight slowing of rate of respiration, in the absence of hyperventilation.

Effect of Prolonged Administration of Diamox Every Six Hours. Patients with pulmonary emphysema and elevated arterial pCO_2: The effect of ingestion of 2.2 to 6.3 mg./kg. of diamox every six hours was studied during eight different courses of therapy in this group of five patients. A course of therapy lasted from three to twelve weeks except in patient B. M., in whom the drug was discontinued at the end of twelve days because of an exacerbation of chronic bronchiectasis.

These studies were not designed to determine when inhibition of acidification of the urine ceased but it was noted that, with few exceptions, alkalinization of the urine no longer occurred after a few days of continued administration of the drug. During the eighth week of therapy patient J. W. excreted an alkaline urine in the interval between the second and fourth hours without an associated sodium, potassium, bicarbonate or chloride diuresis. In this interval the arterial pCO_2 fell from 91 to 82 mm. Hg and plasma bicarbonate level from 33.9 to 32.2 mEq./L. In studies extending over nearly nine weeks in patient J. T. the reaction of the urine consistently changed from acid to alkaline during the four hours following each morning dose of diamox. Only following the initial dose was this accompanied by a sodium, potassium and bicarbonate diuresis and a fall in the level of plasma bicarbonate. (Table II.)

The maximum reduction in the level of plasma bicarbonate following ingestion of the drug occurred fairly promptly and thereafter remained relatively unchanged. The earliest noted occurred within four hours (J. W., Table II). In some instances it was evident within the first two days, in others between the second and seventh days. The sustained reductions in the level of plasma bicarbonate were either highly significant ($P < 0.01$) or significant ($P < 0.05$) in all patients. (Table III.)

In all five patients the plasma chloride level usually rose to replace the fall in plasma bicarbonate. There sometimes was a transient fall in the level of plasma potassium during the first few days of diamox ingestion. The plasma sodium level remained unchanged.

In two patients of this group (B. M., M. A.) the level of arterial pCO_2 was stable and fell significantly ($P < 0.05$) or highly significantly ($P < 0.01$) (Table III) simultaneously with the plasma bicarbonate. Only patient B. M. exhibited a significant reduction in arterial pH due to a relative and absolute greater fall in plasma bicarbonate level than in arterial pCO_2.

A sustained rise in the level of alveolar pO_2 occurred in both these patients along with the fall in arterial pCO_2. This was associated with hyperventilation in patient B. M. Patient M. A. did not exhibit hyperventilation or a change in the graphically recorded respiratory pattern. Neither patient exhibited a rise in arterial blood oxygen saturation while on diamox. Only patient M. A. attained a normal arterial blood oxygen saturation level during inhalation of 100 per cent oxygen

In the three remaining patients in this group (J. W., J. T. and H. R.) the level of arterial pCO_2 often fluctuated considerably above and below control levels from day to day and frequently during the course of the morning and early afternoon of the same day in the absence of change in clinical state and respiratory gas exchange ratio, R.Q. Their mean level of arterial pCO_2 and pH was not significantly altered as compared with the control periods. (Table III.)

Two of these latter patients, J. T. and J. W., offered an opportunity to evaluate the diuretic effect of diamox. J. T., whose congestive heart failure was poorly controlled with digitalis, ammonium chloride, mercuhydrin, aminophylline and a low-salt diet did not exhibit weight loss during seven and one-half weeks of diamox administered every six hours. During the first twenty-three days each dose was 2.3 mg./kg. and from the twenty-fourth day onward each dose was 3.6 mg./kg. He exhibited a biochemical response to the drug as manifested by a prompt fall in the level of plasma bicarbonate from 36.4 to 30.1 mEq./L. which was sustained, with minor fluctuations, throughout the period of administration of the drug. Plasma bicarbonate level had already risen forty-eight hours after discontinuance of diamox and was at the control period level when measured a week later. A gain in weight did not accompany the retention of bicarbonate.

Approximately one month later, when his

plasma bicarbonate level was slightly lower and arterial pCO_2 higher than previously, diamox was given in a larger dose, 4.2 mg./kg. every six hours. He was still edematous and his weight stable at 122 pounds. There was a loss of 2 pounds during the first day and of 8 pounds during the second and third weeks. It remained at this level with minor fluctuations during the next month. The patient exhibited approximately the same sustained fall in plasma bicarbonate during this period of study as he did during the first period when there was no weight loss. Upon discontinuance of the drug, plasma bicarbonate returned to its control level within four days. It seems reasonable to ascribe the sustained loss of weight to diamox for the following reasons: the reaction of the urine became alkaline within two to four hours whenever the effect of a single dose was studied, and during the first week following discontinuance of the drug there was a steady gain in weight from 114 to 119 pounds.

The other patient, J. W., managed to remain edema-free by marked limitation of activity and

TABLE III

STATISTICAL EVALUATION OF CHANGES IN THE ACID-BASE BALANCE DURING LONG-TERM ADMINISTRATION OF DIAMOX

Patient	Dose (mg./kg.)	Days on Diamox	Hours after Dose	Period	No. of Observations	Arterial pCO_2 (mm. Hg)	Plasma $BHCO_3^-$ (mEq./L.)	Arterial pH
A. Patients with Chronic Pulmonary Emphysema								
M. A.				Control	6	60 ±3	30.2 ±1.1	7.33 ±.02
	4.2 × 4	58	4‡	Diamox	5	50* ±2	23.5* ±1.7	7.30 ±.03
B. M.				Control	3	51 ±2	25.5 ±0.8	7.33 ±.02
	6.3 × 4	12	4	Diamox	2	45† ±1	18.0* ±0.4	7.23† ±.00
H. R.				Control	7	74 ±9	33.0 ±1.6	7.28 ±.06
	3.3 × 4	84	4‡	Diamox	4	69 ±7	26.8* ±1.5	7.22 ±.04
J. T.				Control	7	71 ±8	35.7 ±1.3	7.33 ±.06
	4.2 × 4	60	4§‡	Diamox	9	67 ±5	30.2* ±0.7	7.29 ±.04
J. W.				Control	6	87 ±7	39.3 ±2.6	7.28 ±.03
	4.4 × 4	80	4§‡	Diamox	3	77 ±5	31.4† ±1.7	7.24 ±.03
B. Patients without Heart, Lung or Kidney Disease								
C. B.				Control	4	39 ±3	23.1 ±1.5	7.40 ±.02
	3.8 × 4	20	4	Diamox	3	25* ±1	13.2* ±0.1	7.35 ±.02
H. B.				Control	2	46 ±2	26.7 ±1.0	7.40 ±.00
	4.2 × 4	28	4‡	Diamox	3	33* ±1	17.4* ±0.1	7.35* ±.01
A. F.				Control	3	40 ±5	23.7 ±3.0	7.39 ±.03
	3.7 × 4	13	4	Diamox	2	33 ±0	14.7† ±1.1	7.27† ±.03
J. N.				Control	3	42 ±2	25.6 ±1.1	7.41 ±.04
	4.0 × 4	28	4	Diamox	3	34† ±3	17.6* ±0.6	7.34 ±.02
L. S.				Control	2	41 ±6	25.0 ±2.2	7.42 ±.02
	3.7 × 4	18	4	Diamox	2	37 ±5	17.0† ±0.7	7.30 ±.04

Changes of comparable significance in the level of arterial pCO_2, pH and plasma bicarbonate were noted two to three (§) and six (‡) hours after the last dose of diamox.

* $p = <0.01$.

† $p = <0.05$.

a low-salt diet. He experienced a 2- to 4-pound weight loss ascribable to diamox during two of three different periods of study. In each study he experienced a prompt 6 to 9 mEq./L. fall in the level of plasma bicarbonate In the dose range employed (2.2 to 4.4 mEq./L.) there was time the plasma bicarbonate and arterial pCO_2 returned somewhat to their previous levels and the reaction of the urine became acid, the ingestion of a single dose of 4.4 mg./kg. of diamox was followed within four hours by an additional fall of 6.1 mEq./L. plasma bicarbonate, of 3 mm.

TABLE IV

AN EXAMPLE OF HYPERVENTILATION DURING LONG-TERM ADMINISTRATION OF DIAMOX (PATIENT C. B.) *

Day of Study	$\dot{V}_E$ per m^2	f	V_T	$\dot{V}O_2$ per m^2	E	VR_{H,pCO_2}†	$P_{A_{O_2}}$	S_a	pH_a	P_aCO_2	Plasma $BHCO_3^-$ (mEq./L.)	Plasma Cl^- (mEq./L.)	Urine pH
Control													
	4.20	13.2	591	123	.35	0.86	102	95	7.38	37	21.2	103.7	6.37
Diamox (3.8 mg./kg. every 6 hr) ‡													
0	5.27	13.3	739	138	.33	2.99	103	95	7.31	39	19.1	106.0	7.48
7	6.21	18.5	619	117	.33	−0.90	118	96	7.33	26	13.2	107.8	7.01
13	6.79	20.8	602	117	.33	−1.62	119	94	7.35	25	13.3	112.2	6.92
20	7.24	20.1	654	120	.32	−2.10	122	98	7.36	24	13.1	112.2	7.01
Post-diamox													
6	5.08	21.7	428	117	.27	1.54	98	97	7.40	41	24.7	100.4	6.93
12	4.31	18.9	417	116	.27	1.70	94	97	7.39	41	23.9	98.1	6.79

* A similar response was exhibited by patients J. N. and L. S., who, like C. B. did not have heart, lung or kidney disease and B. M., who had chronic bronchiectasis and pulmonary emphysema.

† Alveolar ventilation ratio according to Gray.[28]

‡ Observations carried out 4 hours after a morning dose of diamox.

no relationship between the size of the dose and the absolute fall in plasma bicarbonate level or extent of diuresis.

The immediate response to two individual doses of diamox administered to patient J. W. a week apart resulted in a total fall of 15 mEq./L. plasma bicarbonate, 11 mm. Hg arterial pCO_2 and 0.11 pH units. (Table II.) Within four hours after the first diamox dose of 2.2 mg./kg. there was a 9.0 mEq./L. fall in plasma bicarbonate and an 8 mm. Hg decrease in arterial pCO_2 and a 0.06 decrease in the level of arterial pH. The drug was continued in the same dose every six hours during the following three and one-half days. We do not know whether further change in the acid-base balance of the blood occurred during this interval. Following a diamox-free interval of three and one-half days, during which Hg arterial pCO_2 and of 0.05 pH units. (Table II.) In all, the intermittent administration of the drug resulted in a net fall of 13.7 mEq./L. of plasma bicarbonate (43.8 to 30.1), 15 mm. Hg arterial pCO_2 (94 to 79) and 0.08 pH units (7.29 to 7.21). This was associated with only a 4-pound weight loss.

Patients without lung, heart or kidney disease: All of the patients exhibited a significant or highly significant fall in plasma bicarbonate and, in three instances, also of arterial pCO_2. (Table III.) The level of arterial pCO_2 was stable when measured a few times during a four-hour period following a dose of diamox, once the maximum effects of the drug were attained. The decrease in the level of plasma bicarbonate was relatively greater than that of arterial pCO_2 and in two patients (H. E. and A. F.), it was sufficient

to cause a significant or highly significant reduction in the level of arterial pH. (Table III.) The level of alveolar pO_2 rose in all five patients. In three (C. B., J. N. and L. S.,) (Table IV) this was accomplished by hyperventilation characterized by a greater increase in depth than in rate of breathing. The remaining two patients (H. B. and A. F.,) (Table V) did not exhibit hyperventilation but rather an increase in depth and a slight decrease in rate of breathing. All patients in this group exhibited a normal level of arterial blood oxygen saturation which did not increase when the alveolar pO_2 rose while on diamox.

Acidification of the urine was generally inhibited only during the first few days of drug administration. This was accompanied by a diuresis of sodium, potassium and bicarbonate ions.

Effect of Prolonged Administration of Diamox on the Response of Patients with Chronic Pulmonary Emphysema and Respiratory Acidosis to Inhalation of 100 Per Cent Oxygen. Table VI summarizes nine studies in three of the five patients with chronic pulmonary emphysema during inhalation of oxygen* for forty-four to ninety minutes in the control and diamox periods. The change in minute volume of respiration during oxygen inhalation is expressed as per cent of the average of a seven-minute period of room air breathing preceding oxygen inhalation. In nearly all instances average minute volume of respiration decreased while breathing oxygen. Ingestion of diamox did not influence the response. Decrease in minute volume of respiration was associated in four instances with a relatively greater decrease in tidal volume than rate; in two instances these decreased to the same relative degree and in one, rate decreased more than depth. Tidal volume increased in two studies in

TABLE V

PATIENT H. B.—AN EXAMPLE OF A FALL IN $PaCO_2$ WITHOUT HYPERVENTILATION DURING LONG-TERM ADMINISTRATION OF DIAMOX*

Day of Study	$\dot{V}_E$ per m^2	f	V_T	$\dot{V}O_2$ per m^2	R	VR_{H,pCO_2}†	$P_{A_{O_2}}$	S_a	pH_a	P_aCO_2	Plasma $BHCO_3^-$ mEq./L.	Plasma Cl^- mEq./L.	Urine pH
Control													
	4.09	17.6	409	103	.83	....	...	..	....	..	....	.....	....
	4.52	17.0	467	107	.84	2.29	99	96	7.40	44	26.0	103.5	6.60
Diamox (4.2 mg./kg. every 6 hours)‡													
0	5.04	18.8	472	120	.83	1.87	103	96	7.37	40	22.0	105.0	7.48
5	5.03	15.1	574	120	.85	0.74	110	95	7.35	34	18.2	113.0	7.10
12	5.34	14.8	624	127	.85	0.97	111	95	7.34	34	17.2	111.0	7.06
25	5.19	16.2	551	123	.85	0.21	111	97	7.35	32	16.9	111.6	6.80
Post-diamox													
7	4.16	17.1	418	106	.85	3.07	96	94	7.40	47	27.4	.....	6.51

* Note that the patient exhibited a moderate increase in tidal volume (V_T) with respiratory rate generally remaining the same or slightly slower during the diamox period. A similar response was exhibited by patient A. F., who, like H. B., did not have heart, lung or kidney disease, and patient M. A., who suffered from chronic pulmonary emphysema.

† Alveolar ventilation ratio according to Gray.[28]

‡ Observations carried out four hours after a morning dose of diamox.

* The composition of the gas delivered from commercial tanks varied between 98.95 and 99.16 per cent oxygen and 1.05 to 0.84 per cent nitrogen.

patient H. R. along with a decrease in respiratory rate during the diamox period. Rate of respiration and tidal volume generally varied in opposite directions. In no instance did rate of respiration exceed that while on room air. Periodic respirations characterized by fluctuations in tidal volume and rate of respiration were common. Only patient J. W. exhibited apneic periods, which lasted fifteen to twenty-five seconds. These occurred during one of each of the two studies in the control (April 6, 1953) and in the diamox (July 13, 1953) periods.

There was no over-all consistent relationship between the degree of respiratory depression exhibited by these patients during periods of oxygen inhalation and any of the following: the initial level of plasma bicarbonate and the extent to which it fell under influence of diamox or rose during oxygen inhalation, the initial level of arterial pCO_2 and pH, or the level to which arterial blood oxygen saturation rose. (Table VI.) The level of plasma bicarbonate rose 1.4 to 3.3 mEq./L. in three of five studies on oxygen during control periods and 3.0 and 3.4 mEq./L. in two of six studies during diamox periods. (Table VI.) No patient in whom more than one study was carried out during either control or diamox periods exhibited a compensatory rise in plasma bicarbonate more than once in either period.

Arterial blood oxygen saturation varied between 57 and 80 per cent when breathing room air. It rose to normal in only one patient, M. A., and remained unchanged or increased to between 71 and 92 per cent in the other two patients. It seems reasonable to expect that the level of arterial blood oxygen saturation would stabilize after many minutes of oxygen inhalation in spite of periodic or depressed respirations, whereas the arterial pCO_2 level would vary considerably. The arterial pCO_2 during oxygen inhalation periods listed in Table VI reflects only the level during the intervals of blood withdrawal. This limitation no doubt also applies to arterial pH but to a varying degree depending upon accompanying changes, if any, in plasma bicarbonate level. The acidosis was more intense during inhalation of oxygen in those instances in which fall in plasma bicarbonate during the diamox period was unaccompanied by a significant fall in the level of arterial pCO_2. None of the patients manifested mental or neurologic changes during periods of oxygen inhalation even though there were instances of a considerable rise in the level of arterial pCO_2 and intense acidosis.

DISCUSSION

Immediate Effects of a Single Dose of Diamox in Patients with Chronic Pulmonary Emphysema and in Patients without Lung, Heart and Kidney Disease. The immediate alterations in urinary electrolyte excretion following an oral dose of diamox have been described in detail.[4,11,22,23] The onset of diuresis of sodium, potassium, bicarbonate and water may be noted within about an hour, is apt to reach its peak at approximately ninety minutes, may be complete by four to five hours and may be over in eight hours.[22] The present studies were designed to detect correlated changes in respiration, arterial blood acid-base balance, plasma electrolyte levels and urinary electrolyte excretion during the four-hour interval when the drug exerts most of its effects.

Our patients exhibited considerable variation in the fall of plasma bicarbonate level in response to a single dose of diamox. (Table II.) The responses of patient J. W. suggest that it varies with the initial plasma bicarbonate level but the data on all the patients do not bear out this relationship, nor do the initial levels of arterial pCO_2, oxygen saturation or of pH seem to be closely related. With intravenous administration of diamox in dogs, bicarbonate diuresis is directly proportional to the initial plasma bicarbonate concentration.[24] The response of our patients to oral administration of the drug does not reflect this nor does the intensity of the diuresis vary with the absolute decrease in the level of plasma bicarbonate. (Table II.) Our studies and those of Schwartz et al. are obviously not strictly comparable because of the differences in species studied and because the dietary intake of our patients was not rigidly controlled.

The urine of all of our patients became alkaline between the first and third hours and this was associated with a sodium, potassium and bicarbonate diuresis. Others have demonstrated that in addition to these alterations in urinary electrolyte excretion there is a decrease in titratable acidity and in ammonium ion excretion,[11,23] and a slight[11] or no increase in urinary phosphate.[23] Chloride ion excretion generally remains unchanged.[11] In some instances it rises.[23] Excretion of nitrogen and organic acids remains unchanged.[23]

Although all patients exhibited some fall in the level of plasma bicarbonate, varying from

TABLE VI

STUDIES ON THE RESPIRATORY* AND ACID-BASE BALANCE ADJUSTMENTS TO OXYGEN INHALATION IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA DURING CONTROL AND DIAMOX PERIODS

Patient	M. A.				H. R.						J. W.							
Period	Control		Diamox		Control		Diamox				Control				Diamox			
rO_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2	Rm Air	100% O_2
Dose (mg./kg.)			4.2				3.3		3.5						2.2		4.4	
Date	6/17/54		4/23/54		5/5/54		7/15/53		4/27/54		4/2/53		4/6/53		5/8/53		7/13/53	
Time on 100% O_2*	58′		58′		45′		45′(67′)		45′		65′(74′)		65′		65′		65′(90′)	
$\dot{V}_E$	7.35	−14.5	7.68	−7.9	6.98	−12.9	8.54	−19.0	6.74	−0.4	7.04	−44.1	5.73	−31.3	6.46	−46.2	6.24	−42.6
V_T	417	−12.3	423	−7.1	342	−6.7	339	+3.4	316	+12.8	371	−27.9	356	−26.0	366	−25.5	365	−18.5
f	17.6	−2.5	18.1	−0.7	20.4	−6.3	25.2	−21.6	21.8	−11.8	19.0	−21.6	16.1	−6.6	17.6	−25.0	17.1	−28.4
S_a	80.4	98.6	77.4	96.7	65.9	83.7	72.2	92.3	62.9	83.4	62.8	84.0	56.8	74.0	64.0	83.7	68.2	71.3
pH_a	7.34	7.31	7.31	7.29	7.30	7.18	7.24	7.19	7.23	7.17	7.28	7.18	7.27	7.09	7.20	7.05	7.21	7.05
P_aCO_2	61	69	51	55	64	88	69	78	76	88	81	110	87	133	83	127	88	127
Plasma $BHCO_3^-$(mEq./L.)	31.7	33.8	25.1	25.8	30.5	31.9	28.1	28.3	30.7	31.2	35.8	39.1	38.2	38.2	31.0	34.1	34.1	34.1
Plasma Cl^-(mEq./L.)	95.7	95.0	103.9	107.0	95.7	93.6	102.8	102.1	101.5	102.5	95.5	95.8	92.2	94.5	....	.....	103.1	101.9

* The respiratory data during 100 per cent oxygen inhalation in the control and diamox periods are based upon the average values of intervals of the same number of minutes. They are expressed as per cent increase (+) or decrease (−) as compared with a preceding seven minute room air period. The minutes enclosed in parentheses indicate instances where oxygen inhalation was continued for longer periods of time. There were no significant changes in the average values over these more prolonged periods. Arterial blood samples were collected during the second to fifth minutes of the last seven minutes of oxygen inhalation. The graphic respiratory tracings were measured minute by minute. Only patient J. W. exhibited Cheyne-Stokes breathing while inhaling oxygen. He exhibited apneic intervals of 15 to 25 seconds in the first fifteen minutes of oxygen inhalation on April 6, 1953 and in the seventh, fifty-fourth and fifty-eighth minutes on May 8, 1953 and at no time during blood collection.

0.7 to 9.0 mEq./L. (generally 2 to 4 mEq./L.), within four hours of ingestion of a dose of diamox, the level of arterial pCO_2 either remained essentially unchanged or fell 4 to 8 mm. Hg. In one patient, L. S., it was 7 mm. Hg higher. Arterial pH either remained unchanged or fell 0.04 to 0.10 units, in these instances generally reflecting the relatively greater fall in plasma bicarbonate.

In those instances in which plasma bicarbonate and arterial pCO_2 fell simultaneously within four hours after a single dose of diamox, a moderate rise in alveolar pO_2 and minute volume of alveolar ventilation occurred. This was associated with one of two different alterations in the pattern of respiration. Some patients exhibited a moderate degree of hyperventilation (an increase in ventilation in excess of metabolic needs) presumably stimulated by the fall in plasma bicarbonate. In others, more effective alveolar ventilation appeared to be accomplished by a moderate increase in depth and slight slowing in rate of respiration in the absence of compensatory hyperventilation. Review of Roughton's studies on the response of healthy adults to a single dose of sulfanilamide which as pointed out previously is a less potent carbonic anhydrase inhibitor than diamox, indicates that some of them likely responded in this latter manner.[2]

Effect of Prolonged Administration of Diamox Every Six Hours to Patients with Pulmonary Emphysema and Elevated Arterial pCO_2 and to Patients without Lung, Heart and Kidney Disease. The return of the urinary electrolyte excretion pattern to control period levels within two to three days and its persistence with continued administration of diamox a few times a day has been well documented.[11,22,23] Although we did not carry out metabolic balance studies, some data confirming the transitory nature of the alterations in urinary electrolyte excretion were obtained. A few-fold increase in the dose of diamox does not alter this situation. Unresponsiveness to the drug may, however, appear after the initial dose.[23] This was evident with regard to decrease in the level of plasma bicarbonate in patient J. W., who exhibited as great a fall in plasma bicarbonate following the initial dose of diamox in each of two different courses of therapy as he did during days to weeks of continuous ingestion of the drug. His urine was not examined at sufficiently frequent intervals to note whether the renal effects of the drug were short-lived.

Friedberg and his co-workers[22] and also Belsky[25] reported continued effectiveness of a single daily dose of 0.25 to 0.5 gm. of diamox based on loss of body weight in the treatment of patients suffering from congestive heart failure in the outpatient department. In contrast, in carefully controlled metabolic studies in hospitalized patients suffering from congestive heart failure Leaf[23] and Relman[26] observed fairly prompt refractoriness to the drug when given once daily or every other day.

Another exception to the usual transient response to the drug is the observation that urinary acidification may continue to be inhibited for eight and one-half weeks (4.2 mg./Kg. every six hours, patient J. T.) and for as long as four weeks of a twenty-week course of a daily dose (3.5 mg./kg., patient H. R.). Only during the first few days of these two studies was alkalinization of the urine associated with sodium, potassium and bicarbonate diuresis. Leaf also noted inhibition of urinary acidification without sodium diuresis in three patients with congestive heart failure.[23] In two of these patients there was an equivocal or slight increase in urinary potassium, associated with water diuresis in only one instance. A definite potassium diuresis occurred in two different studies in the third patient.

It is generally agreed that a reduction in plasma bicarbonate level accompanies the urinary electrolyte diuresis.[27] By the second to the seventh day of continued administration of the drug, peak reduction in the level of plasma bicarbonate of about 5 to 10 mEq./L. below the control level occurs.[11] Plasma chloride often rises to approximately the same degree as the bicarbonate level falls, resulting in hyperchloremic acidosis. Although the diuresis is transient the reduced level of plasma bicarbonate persists.

There is, however, conflict in the evidence concerning the adjustment of the acid-base balance of the blood to long-term ingestion of the drug. Leaf observed sustained hyperchloremic acidosis for as long as eleven days in normal subjects and in patients with pulmonary emphysema and respiratory acidosis.[23] Nadell noted in similar groups of patients a return of the acid-base balance of the blood to normal associated with a fall in the level of arterial pCO_2 after one to two weeks of treatment.[11] The fall in arterial pCO_2 was logically attributed to compensatory hyperventilation stimulated

by the acidosis which resulted from the diamox-induced fall in plasma bicarbonate. This led to the conclusion that diamox brings about more effective dissipation of carbon dioxide in the lungs.[11] It also indicates delayed onset of an altered sensitivity of the respiratory regulatory mechanism to a decrease in the level of plasma bicarbonate.

However, measurements of minute volume of respiration, oxygen consumption and respiratory gas exchange ratio (R.Q.), which appear essential to establish whether the delayed fall in the level of arterial pCO_2 actually reflects metabolically induced conpensatory hyperventilation, were not carried out. A transitory fall of a few millimeters Hg in the level of arterial pCO_2 and rise in arterial pH accompanied by little or no fall in plasma bicarbonate may result from moderate hyperventilation induced by the discomforts of an experimental procedure. The influence of such stimuli would no doubt be distinguished by an inconsistency in the results of frequent respiratory measurements along with measurements of arterial blood pH, pCO_2, plasma bicarbonate levels and of arterial blood pressure and pulse rate. Therefore, in our studies such observations were carried out, as described under *Methods*.

In no instance did we observe a lag of a week or two in the fall of arterial pCO_2 behind that of plasma bicarbonate, nor a rise in the mean level of arterial pH to normal in the chronically acidotic patients, nor a return to the control pH values in the patients without lung, heart and kidney disease. However, although the mean arterial pH was lower during the diamox period in all patients, it was significantly reduced in only one of the patients with chronic pulmonary emphysema and in three of the patients without lung, heart and kidney disease. The prompt partial respiratory compensation for the diamox-induced metabolic acidotic component (decrease in plasma bicarbonate) noted by us, rather than a delayed complete compensation, is to be expected according to Gray's studies on the integrated regulation of pulmonary ventilation.[28] Gray points out that "since the factor which initiates and maintains the ventilatory adjustment is the pH change itself, the compensation cannot be sufficient to restore the original pH, for this would eliminate the respiratory stimulus which is driving the compensatory mechanism" (ref. 28, p. 54).

It is known that in normal individuals an acute fall in arterial pH resulting from plasma bicarbonate depletion is a feeble respiratory stimulus as compared to a similar fall in the level of arterial pH resulting from sudden elevation of arterial pCO_2.[28] Patients suffering from pulmonary emphysema associated with carbon dioxide retention exhibit considerably less respiratory stimulation than normal individuals from an acute elevation in the level of arterial pCO_2 upon inhalation of mixtures of carbon dioxide and high oxygen.[29–31] It might therefore be expected that such individuals would be even more insensitive to a fall in arterial pH resulting from a decrease in the level of plasma bicarbonate.

Donald and Christie,[30] and Tenney[31] reported a diminished ventilatory response to carbon dioxide in patients suffering from pulmonary emphysema, to a degree which is proportional to the level of carbon dioxide retention in their blood. Tenney also noted that increased responsiveness to this stimulus developed in these patients one to two days after their blood carbon dioxide content fell as a result of the ingestion of 10 mg./kg./24 hours of diamox.[31] This lag in the onset of increased ventilatory response was ascribed to a probable readjustment of the sensitivity of the respiratory center from adaptation to an elevated blood pCO_2 level.

Tenney suggested that this mechanism might account for the delay of one to two weeks in the fall of arterial pCO_2 in patients with chronic pulmonary emphysema and respiratory acidosis treated with diamox by Nadell.[11] However, since it is known that the respiratory center of normal individuals is exquisitely sensitive to minor alterations in arterial pH and pCO_2 it is difficult to involve the same reasoning to explain the similar lag noted in normal subjects by Nadell.

Although Fishman et al. also noted a return of arterial pCO_2 and plasma bicarbonate to normal levels in patients with chronic pulmonary emphysema and carbon dioxide retention treated with diamox, these patients did not then, contrary to Tenney's findings, exhibit an increased responsiveness to inspired CO_2.[32] The response of these patients was not influenced by the level of arterial hypoxemia, pH or red blood cell mass and reduced ventilatory capacity was excluded as a cause for failure of ventilation to increase. It was concluded that such patients suffer from permanent injury to the respiratory center. These studies did not, how-

ever, account for the manner in which the level of arterial pCO_2 fell. Our studies, as has previously been discussed, indicate that this may come about by different patterns of ventilatory adjustment which have a common net effect. This was noted in both groups of patients studied.

It is of interest to analyze the respiratory adjustments of our patients to carbonic anhydrase inhibition according to Gray's concepts of the integrated chemical regulation of pulmonary ventilation.[28] Gray derived a multiple linear equation* which describes quantitatively the partial effects of changes in arterial blood H^+ concentration, pCO_2 and pO_2 on ventilation. It involves the assumption of an equality in the levels of arterial and alveolar pCO_2 and pO_2. It is known that such relationships do not exist in the presence of pulmonary emphysema. The formula may therefore be used with confidence only in those of our patients without lung, heart and kidney disease. Variations in the level of arterial pO_2 above 80 mm. Hg have no effect on respiration in this formula. Although we did not measure arterial pO_2 directly, the values derived by interpolation from the oxygen dissociation curve fell above the 80 mm. Hg level in all our patients without lung, heart and kidney disease. The changes in the level of arterial pO_2 may therefore be disregarded in these patients.

When Gray's formula is applied in this manner to instances of metabolic acidosis, it can be seen that an increase in H^+ concentration due to plasma bicarbonate depletion stimulates respiration. This leads to a fall in arterial pCO_2. The fall in the level of arterial pCO_2 depresses respiration. The net effect of these two opposing respiratory stimuli is an increase in ventilation in excess of metabolic requirements, reflected by a greater VR_{H,pCO_2} ratio as compared with the pre-acidotic state.

Under the influence of prolonged administration of diamox, the alveolar ventilation ratio, VR_{H,pCO_2}, of our patients falls below that of the control period (Tables IV, V). This indicates that the inhibitory effect on ventilation of the decreases in the mean level of arterial pCO_2 exceeded the stimulatory effect of the relatively minor decreases in the mean levels of arterial pH. Had ventilation been regulated in these patients during the diamox periods solely by these chemical stimuli, according to Gray's views, it would have ceased or been depressed as compared with the control periods.[28] The fact that the measured volumes of alveolar ventilation increased in all patients in whom the mean level of arterial pCO_2 fell significantly indicates, according to Gray's concepts, that some other respiratory stimulus must have been operating. Whether this is due to direct or reflex stimulation of the respiratory center by carbonic anhydrase inhibition is not evident.

The fact that in all instances the changes in respiration noted by us no longer occurred when the level of arterial pCO_2 and plasma bicarbonate returned to control levels following cessation of diamox therapy makes it seem likely that they were causally related to the effects of the drug.

Three of the five patients (J. W., J. T., H. R.) with advanced pulmonary emphysema and respiratory acidosis exhibited considerable fluctuations in the level of arterial pCO_2 from day to day and during the morning and early noon of the same day in the absence of change in clinical manifestations or in minute ventilation and respiratory gas exchange ratio (R.Q.). (Table III.) The evidence indicated that spontaneous variations in effectiveness of alveolar ventilation were occurring without adequate explanation. These findings serve to emphasize the limitations of ascribing a decrease in the level of arterial pCO_2 to diamox in such patients and the importance of statistical evaluation of their significance.

Studies on the Effect of Prolonged Administration of Diamox on the Response of Patients with Chronic Pulmonary Emphysema and Respiratory Acidosis to the Inhalation of 100 Per Cent Oxygen. It is currently believed that the respiratory drive of patients suffering from pulmonary emphysema associated with anoxemia and carbon dioxide retention is maintained by anoxic reflex stimuli arising in the carotid and aortic bodies. Acute relief of anoxemia by oxygen inhalation causes a suppression of the respiratory drive. Minute volume of respiration therefore decreases and as a result the level of arterial pCO_2 rises. Mental and neurologic symptoms develop in

* In this equation $VR_{H,pCO_2,pO_2} = 0.22H + 0.262\ pCO_2 - 18 + 2.118 \times 10^{-8}\ (104 - pO_2)^{4.9}$. VR refers to alveolar ventilation ratio. This is the alveolar ventilation expressed as a multiple of the resting ventilation. The subscript symbol, H, represents the H ion concentration of arterial blood expressed in billionths of moles per liter and similarly pCO_2 and pO_2 refer to their tensions in arterial blood.[28]

about 10 per cent of these patients during oxygen inhalation, for reasons which are not fully understood.[13] There is disagreement concerning the biochemical changes in the blood which predispose to these potentially serious complications. Some have indicated that the primary factor is a sharp fall in arterial pH due to failure of plasma bicarbonate level to rise along with the increase in arterial pCO_2.[12,13] Comroe has emphasized that the elevated arterial pCO_2 level is the determining factor.[13] It was believed that some data which bear upon both these concepts might be obtained by comparison of the respiratory, biochemical and clinical responses to inhalation of 100 per cent oxygen, before and during diamox administration, in the patients with pulmonary emphysema who exhibited only a significant fall in plasma bicarbonate and in the patients who, in addition, exhibited a fall in arterial pCO_2 during the diamox period. According to the views of Barach[12] and Richards,[33] a decrease in the level of plasma bicarbonate, unaccompanied by a fall in arterial pCO_2 during the diamox period, might be expected to increase the severity of respiratory depression during inhalation of oxygen and predispose to the development of mental symptoms and neurologic disturbances. This would be more likely to occur in those instances in which a compensatory rise in plasma bicarbonate did not take place during oxygen inhalation. It was a fortunate coincidence that the level of arterial pCO_2 and arterial blood oxygen saturation varied very little in certain groups of studies (Table VI) carried out during the control and diamox periods in patient J. W., which made it possible to evaluate this concept. It is evident that this patient did not exhibit a greater decrease in minute volume of respiration on oxygen following intensification of acidosis due to a fall in plasma bicarbonate level during the diamox period. Similarly, failure of plasma bicarbonate level to rise during oxygen inhalation in the control and diamox periods did not alter the degree of respiratory depression. In no instance did the patient develop mental and neurologic symptoms.

The studies of Comroe et al.[13] suggest a potential beneficial effect of diamox in the prevention of mental symptoms during inhalation of oxygen in patients in whom it brings about a reduction in arterial pCO_2 to less than 50 mm. Hg. They concluded on the basis of studies in sixty-five patients with chronic pulmonary emphysema that mental symptoms occur only in patients with an arterial pCO_2 level of 50 mm. Hg or more, associated with anoxemia, which is relieved by inhalation of oxygen. Unfortunately for the purpose of evaluating the possible beneficial effects of the drug, none of our patients experienced mental symptoms during either the control or diamox periods. It was thought, however, that in keeping with Comroe's view those patients who experienced a significant fall in the level of arterial pCO_2 during diamox therapy might exhibit less respiratory depression during oxygen inhalation. Such studies could be carried out only in one (M. A.) of the two patients who exhibited a significant fall in the level of arterial pCO_2 (from 61 to 51 mm. Hg) and of plasma bicarbonate (from 32 to 25 mEq./L.) during diamox therapy. Arterial blood oxygen unsaturation was also relieved in this patient by oxygen inhalation. (Table VI.) He did not exhibit less respiratory depression during oxygen inhalation in the diamox period than during the control period.

A potential disadvantage resulting from the use of diamox was exhibited by patient J. T. when bronchopneumonia developed complicated by a rise in the level of arterial pCO_2 and a fall in the level of plasma bicarbonate during a course of diamox therapy. In the absence of this complication the patient exhibited a fall in the mean level of plasma bicarbonate from 35.2 to 30.9 mEq./L. associated with statistically insignificant changes in the level of arterial pCO_2 and pH. (Table III.) While suffering from bronchopneumonia plasma bicarbonate level fell an additional 4.9 mEq./L., i.e. to 26 mEq./L., and arterial pCO_2 rose to 100 mm. Hg and arterial pH fell to 7.05. It can be estimated by the graphic method of Davenport[34] that for this rise in the level of arterial pCO_2 arterial pH would have fallen to only 7.20, if one makes the reasonable assumption that in the absence of diamox ingestion mean plasma bicarbonate level would have fallen from 35.2 mEq./L. to only 30.3 mEq./L. during the bronchopneumonia. Even greater intensification of the acidosis would occur should such a patient experience ventilatory depression and carbon dioxide retention during oxygen therapy, which is commonly administered in the presence of bronchopneumonia. It is, of course, likely that partial compensation for the increase in arterial pCO_2 would occur as a result of increased renal bicarbonate

reabsorption stimulated by the rising arterial pCO_2.[35–37]

Another potential disadvantage in the use of diamox, should clinical complications associated with either carbon dioxide retention or plasma bicarbonate depletion, or both, develop is the delay of about three to five days in the rise of plasma bicarbonate to its usual level following discontinuance of the drug.

The marked sensitivity of the respiratory regulatory mechanism of patients with chronic anoxemia to only minor increases in arterial blood oxygen saturation is illustrated by the studies carried out in patient J. W. A rise of only 3 per cent during oxygen inhalation on July 13, 1953, was associated with a 42.6 per cent decrease in minute volume of respiration. (Table VI.) Similarly on April 6, 1953, when he exhibited a comparable degree of anoxemia during oxygen inhalation, minute volume of respiration was 31.3 per cent less than when breathing room air. (Table VI.)

These studies demonstrate that the respiratory depression during oxygen inhalation is not of the same intensity in different patients who exhibit comparable degrees of anoxemia in room air and during oxygen inhalation. Patient H. R., starting from approximately the same level of arterial oxygen saturation, attained 84 per cent saturation on April 27, 1954, and May 5, 1954. However, his average minute volume of respiration was depressed 12.7 per cent on May 5, 1954, and only 0.4 per cent on April 27, 1954. (Table VI.) Similarly, patient J. W. attained the same degree of arterial oxygen saturation (84 per cent) on April 2, 1953, and May 8, 1953, associated with about 45 per cent decrease in respiration. (Table VI.) These observations indicate that in these patients with chronic anoxemia the level of arterial blood oxygen saturation is not the sole stimulus for regulation of the minute volume of respiration. It is possible that the presence of heart failure contributed to the greater respiratory depression in patient J. W as compared with patient H. R. In this regard, Comroe et al.[13] reported that of nine chronically anoxemic patients who exhibited mental symptoms during oxygen inhalation, six were in congestive heart failure.

SUMMARY

Studies of the immediate and long-term effects of the ingestion of 2.2 to 6.3 mg./kg. diamox, given every six hours, on acid-base balance of the blood, plasma electrolyte levels, urinary electrolyte excretion rates, respiration and metabolic rate were carried out in five patients suffering from chronic lung disease and respiratory acidosis, and in five patients without lung, heart and kidney disease.

Within four hours after the ingestion of a single dose of diamox both groups of patients exhibited a fall in the level of plasma bicarbonate and alkalinization of the urine with a diuresis of sodium, potassium and bicarbonate ions. The alterations in the level of arterial pCO_2 were variable and arterial pH either remained unchanged or fell moderately. The intensity of these changes bore no relationship to the size of the dose of diamox, the initial levels of plasma electrolytes, arterial pH or oxygen saturation.

Alkalinization of the urine and diuresis of sodium, potassium and bicarbonate no longer occurred after the first few days in all but one patient, who suffered from heart failure associated with chronic pulmonary emphysema and cor pulmonale. He exhibited inhibition of acidification of the urine without electrolyte diuresis throughout an eight and one-half week study period.

Prolonged administration of the drug was accompanied in all the patients by a significant fall in the level of plasma bicarbonate. Arterial pCO_2 fell significantly in three and arterial pH in two of the patients without heart, lung or kidney disease. Two of the emphysema patients exhibited a significant decrease in arterial pCO_2 and one an intensification of acidosis. Arterial pH did not rise to a normal level in the emphysema patients, nor to control values in the patients without lung, heart or kidney disease. Arterial pCO_2 often fluctuated considerably, without apparent cause, in the course of a morning and from day to day in control and diamox periods in three of the chronic emphysema patients.

Hyperventilation or an increase in tidal air and a moderate slowing in rate of respiration, while minute volume of respiration remained relatively unchanged, accounted for the instances of sustained decrease in arterial pCO_2, rise in alveolar pO_2 and alveolar ventilation.

The ventilatory adjustments to prolonged administration of diamox appear to involve a stimulus or stimuli other than those included in Gray's multiple chemical factor theory of the regulation of ventilation.

Intensification of acidosis by diamox-induced fall in plasma bicarbonate in the chronic emphysema patients did not alter their degree of respiratory depression during inhalation of 100 per cent oxygen and the response of one such patient was not less depressed when there was a concomitant reduction in the level of arterial pCO_2.

Potential disadvantages to the administration of diamox every six hours in some patients with chronic respiratory acidosis are intensification of the acidosis due to depletion of plasma bicarbonate and a delay of a few days in its return to control levels following discontinuance of the drug.

Acknowledgment: The author wishes to thank Miss Thora Lee, Dr. Murray Hunter and Dr. Judith Nadell for assistance in the early phase of the studies.

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Ventilatory Drive in Chronic Pulmonary Emphysema*

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In patients with chronic, diffuse, obstructive pulmonary emphysema, a disease characterized anatomically by narrowing of the bronchioles and loss of pulmonary elasticity,[1] the capacity to increase minute ventilation ($\dot{V}_E$)† is an important compensatory mechanism for disturbed alveolar ventilation-perfusion relationships, which constitute a fundamental physiologic aberration of this disease. The defects in distribution, physiologically equivalent to augmented dead space ventilation and venous admixture, would in the absence of compensatory mechanisms impede the elimination of CO_2 from the blood, thereby damming up CO_2 in the large tissue-fluid reservoir; the retention of CO_2 would, in turn, lead to an increase in alkali reserve mediated by the kidney. The respiratory mechanisms available to counteract the tendency to CO_2 retention include (1) the ability of the respiratory center to augment the ventilatory drive, thereby promoting a larger $\dot{V}_E$ and effective alveolar ventilation ($\dot{V}_A$), and (2) the capacity of the chest bellows to respond to an augmented ventilatory drive.

It has long been known[2–6] that in some patients with chronic pulmonary emphysema there is only a slight increase in $\dot{V}_E$ when CO_2 is added to inspired air. This limited ventilatory response has been ascribed to (1) reduction of the maximum ventilatory capacity, (2) increased buffering capacity of arterial blood and (3) depression of the responsiveness of the respiratory center to the stimulus of an increased partial pressure of CO_2 in arterial blood. The potential role of this last mechanism is suggested by experimental observations which demonstrate that in dogs variations in P_{CO_2}, hydrogen ion concentration and alkali reserve of arterial blood and cerebrospinal fluid influence the responsiveness of the respiratory center to the CO_2 stimulus. The therapeutic implication of these observations prompted Boutourline-Young and Whittenberger[9] to attempt restoration of the sensitivity of the respiratory center to the P_{CO_2} stimulus in two patients with chronic pulmonary emphysema and CO_2 retention. For this purpose they used controlled, mechanically-induced, prolonged hyperventilation in order to effect a reduction in arterial blood P_{CO_2} and alkali reserve.

The present study was planned with the following objectives: (1) to identify physiologically the type of patient with chronic pulmonary emphysema in whom there is a reduced ventilatory drive; (2) to determine the mechanisms responsible for the limited increase in $\dot{V}_E$ during CO_2 breathing in these patients, and (3) to evaluate, in patients with chronic pulmonary emphysema and long standing CO_2 retention, the effect on the regulation of respiration of a reduction in arterial blood P_{CO_2} and alkali reserve achieved by the use of a carbonic anhydrase inhibitor. These three aspects of the present investigation are of considerable importance in the medical management of individual patients with this disease.

SUBJECTS FOR STUDY

To facilitate this presentation the patients are classified into three homogeneous groups and a

† Abbreviations are in accordance with the suggestions of the Committee on Standardization of Symbols in Respiratory Physiology. *Fed. Proc.*, 9: 602, 1950.

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fourth miscellaneous group. The first group consists of eleven hospital "normal" subjects free of cardiac or pulmonary disease serving as controls. The second group consists of twelve patients with chronic pulmonary emphysema without CO_2 retention at the time of study and without a medical history suggestive of previous CO_2 retention; these patients will be referred to as patients with emphysema without CO_2 retention. The third group consists of thirteen patients with chronic pulmonary emphysema complicated by CO_2 retention and arterial hypoxemia; these patients will be referred to as patients with emphysema with CO_2 retention. The diagnosis of chronic pulmonary emphysema was established in each patient in accord with criteria previously described.[4] The fourth group consists of six patients with either cardiac disease or pulmonary disease other than chronic pulmonary emphysema and includes two patients (C. S., B. B.) with congestive heart failure, two patients (N. P., G. L.) with secondary polycythemia due to congenital heart disease, one patient (W. W.) with severe primary anemia (6 gm. of hemoglobin per 100 ml. of blood) and one patient (E. H.) with diffuse pulmonary granuloma. Hence five of the six patients had one physiologic abnormality, that is, either polycythemia, chronic hypoxemia, anemia or slowed circulation which might *per se* alter the ventilatory response to inspired carbon dioxide, while the sixth had a chronic pulmonary lesion quite different from emphysema. Pertinent statistics concerning all control subjects and patients are included in Table 1.

The control subjects were studied following recovery from their illness prior to discharge from the hospital. Most of the patients with chronic pulmonary emphysema were ambulatory. Since clinical recognition of an acute upper respiratory infection may be difficult in patients with chronic pulmonary emphysema, particular care was exerted not to study any patient during an overt exacerbation of pulmonary disease. The data in Table 1 were obtained after a period of training and adaptation to the procedures, to the personnel and to the laboratory surroundings; this period of training was required in order to obtain consistent and reproducible ventilatory responses during control and experimental periods. Patients who had been previously studied in the laboratory required little or no training; others required one or more training periods before they could tolerate concentrations of 1 to 5 per cent CO_2 in inspired air without anxiety and with satisfactory evidence of stabilization of the respiration and circulation, such as constancy of ventilation, heart rate, oxygen uptake and respiratory exchange ratio.

All patients were continued on regular treatment schedules. For the emphysema patients without CO_2 retention this was largely confined to inhalations of bronchodilator sprays. The emphysema patients with CO_2 retention were, in addition, usually on low-salt diets and were receiving maintenance doses of digitalis and mercuhydrin® in accord with their clinical needs; seven patients in this group were also treated with diamox,® a carbonic anhydrase inhibitor,* the general trend of the disease in these patients had been documented in this laboratory by repeated ventilatory studies and arterial blood analyses during periods of observation extending up to four years.

METHODS

All patients were studied in the postabsorptive basal state without preliminary sedation. The series of gas mixtures routinely used were 0, 1, 3 and 5 per cent CO_2 in air. Each mixture of CO_2 in air was breathed for twelve to twenty minutes and samples of gas for analysis were collected during the last one and one-half to two minutes of each period. At least twenty minutes of breathing of room air elapsed between runs. This time schedule was based on the demonstration both in normal subjects and in patients with emphysema that (1) ventilation reached a constant level after approximately ten minutes of 5 per cent CO_2 breathing (Fig. 1), (2) arterial blood P_{CO_2}, pH and oxyhemoglobin saturation also reached a plateau at ten minutes and (3) arterial blood P_{CO_2}, pH and oxyhemoglobin saturation had returned to control levels by the twentieth minute of recovery on ambient air. The purpose of the preliminary studies and the experimental plan was to reach and maintain a "steady state" of respiration and circulation[10] and a stable gaseous composition of tissue fluids (particularly with respect to CO_2) so that arterial blood P_{CO_2} and pH might reflect the chemical stimuli at the level of the respiratory center.

* Diamox, the trade name for acetazoleamide, was made available through the courtesy of Dr. James D. Gallagher, Lederle Division of the American Cyanamid Co., Stamford, Connecticut.

TABLE I

VENTILATORY RESPONSE TO BREATHING 5 PER CENT CO_2 IN AIR IN FOUR GROUPS

Subject	Age	Sex	B.S.A.	Breathing Mixture: Ambient Air			Breathing Mixture: 5 Per cent CO_2 in Air		
				$\dot{V}_E$* (L./min.)	f† (per min.)	V_T‡ (ml.)	$\dot{V}_E$ (L./min.)	f (per min.)	V_T (ml.)
Group I: Control Subjects									
E. Mc.	52	M	1.79	9.38	19	493	31.90	18	1773
E. C.	22	M	1.74	7.59	17	467	18.40	16	1150
A. O.	55	M	2.02	9.47	17	557	25.90	17	1524
E. A.	48	M	1.88	10.70	22	486	25.00	22	1135
J. A.	19	F	1.39	7.05	22	321	16.40	20	821
W. M.	32	M	1.91	10.90	20	545	29.30	28	1050
J. K	38	M	1.87	10.80	16	672	30.00	16	1875
A. F.	57	M	1.71	7.58	13	583	16.10	17	945
W. S.	20	M	1.53	9.20	10	920	35.40	18	1965
M. C.	42	F	1.46	5.37	11	523	17.70	16	1104
B. L.	30	M	1.74	9.59	24	460	35.40	22	1610
Average				8.92	17	542	25.59	19	1357
Standard deviation				1.67	5	147	7.42	4	392
Group II: Patients with Chronic Pulmonary Emphysema without CO_2 Retention									
G. E.	63	M	1.71	10.10	26	389	27.30	29	941
G. P.	65	M	1.57	13.40	28	478	30.00	28	1072
P. G.	54	M	1.47	6.50	7	928	17.20	14	1226
J. H.	67	M	1.53	11.00	18	638	23.70	26	910
J. T.	75	M	1.63	7.20	20	350	15.90	18	883
L. T.	39	M	1.91	8.24	12	697	21.20	17	1247
J. S.	39	M	1.69	8.15	19	428	16.20	20	809
T. Ba.	57	M	1.69	8.40	9	933	33.40	15	2228
W. E.	45	M	1.88	12.20	23	531	24.50	23	1065
J. C.	39	M	1.73	9.51	17	550	28.70	27	1062
T. Br.	59	M	1.81	10.10	20	505	20.30	24	846
J. E.	51	M	1.62	8.00	16	500	19.20	19	1038
Average				9.40	18	576	23.13	22	1111
Standard deviation				2.06	2	139	5.77	5	378
Group III: Patients with Chronic Pulmonary Emphysema with CO_2 Retention									
A. P.	53	M	1.57	8.12	24	338	10.40	21	495
A. De.	59	F	1.53	6.92	18	385	7.50	20	375
A. Da.	36	M	1.64	7.28	15	486	13.10	17	771
J. Bu.	45	M	1.47	10.20	22	474	16.60	25	664
A. W.	67	M	1.48	11 50	28	404	19.60	34	576
A. Y.	42	M	1.55	7 25	20	363	10.10	19	532
W. B.	49	M	1.59	10 70	35	306	14.40	31	465
P. M.	52	M	1.70	11.30	28	404	16.90	32	528
B. B.	60	M	1.66	9.74	28	348	17.30	28	614
S. R.	48	M	1.72	9.74	28	348	15.00	40	375
F. P.	63	M	1.76	10.30	20	515	16.30	20	816
J. Be.	58	M	1.59	9.95	20	498	20.40	24	850
J. S.	67	M	1.56	8.30	25	333	14.00	35	400
Average				9.33	24	400	14.74	27	574
Standard deviation				1.47	5	69	3.76	7	162
Group IV. Miscellaneous Patients§									
W. W.	48	M	1.73	8.28	21	394	25.20	20	1260
C. S.	48	M	1.92	11.60	32	363	26.50	27	983
B. B.	63	M	1.88	13.50	35	386	27.40	34	807
N. P.	20	M	1.75	10.20	18	566	39.60	22	1800
G. L.	15	M	1.55	9.09	20	455	35.50	28	1268
E. H.	20	M	1.67	10.80	22	491	37.70	34	1108

* $\dot{V}_E$ = minute ventilation B.T.P.S.
† f = respiratory frequency.
‡ V_T = tidal volume.
§ For diagnosis see text.

The inspired gas mixtures used are indicated in the tables and illustrations. They were administered through an open circuit, using demand valves or an anesthesia bag. Expired gas was collected and measured in a Tissot spirometer from which gas samples were obtained for analysis. Arterial blood was withdrawn anaerobically from an indwelling brachial artery needle during the middle minute of the period of collection of expired gas.

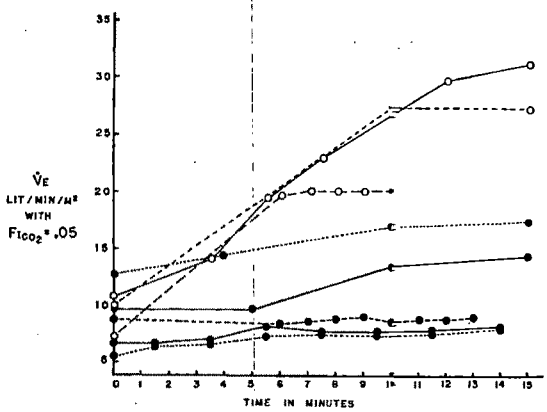


FIG. 1. Graphic representation of the time required for reaching a constant level of ventilation while the patient is breathing a mixture of 5 per cent CO_2 in air; control subjects are indicated by open circles, and patients with pulmonary emphysema and CO_2 retention by closed circles.

All samples were checked in duplicate. The composition of inspired and expired gas was determined using a micro-Scholander apparatus. From these values were calculated the O_2 uptake ($\dot{V}_{O_2}$), CO_2 production ($\dot{V}_{CO_2}$) and respiratory exchange ratio (R_E). Arterial blood samples were analyzed immediately after withdrawal by the method of Van Slyke-Neill for O_2 and CO_2 content and O_2 capacity. Arterial blood pH was determined at constant temperature of 37°C. using the MacInnes-Belcher glass electrode. Arterial blood oxygen tension (P_{aO_2}) was derived from the standard oxyhemoglobin dissociation curve using the value for oxyhemoglobin saturation corrected for pH. The arterial blood carbon dioxide tensions (P_{aCO_2}) were calculated from the line charts of Van Slyke and Sendroy, occasionally supplemented by direct determinations using the bubble method of Riley. The alkali reserve (T_{40}) was expressed as the CO_2 of whole blood at P_{CO_2} of 40 mm. Hg and was obtained from the nomogram of Henderson.

Minute alveolar ventilation ($\dot{V}_A$) was determined as the difference between total minute ventilation ($\dot{V}_E$) and dead space minute ventilation ($\dot{V}_D$). The volume of the respiratory dead space (V_D) for each subject was calculated from the Bohr formula, substituting arterial blood P_{CO_2} for alveolar P_{CO_2}. The measurement of the maximum ventilatory capacity and standard step exercise tests were carried out as previously described.[11] Steady state leg exercise was performed with the patient in the supine position using a pulley system by means of which each leg alternately moved an attached weight a fixed distance.

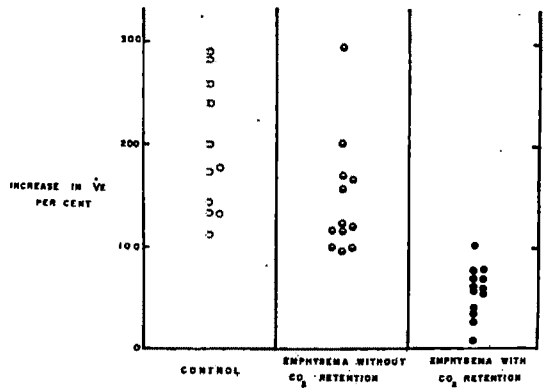


FIG. 2. Chart of the increase in minute ventilation while the patient is breathing a mixture of 5 per cent CO_2 in air in three groups of subjects; for description see text.

RESULTS

1. The ventilatory response to inspired carbon dioxide. The patients were exposed to inspired gas mixtures of 1 per cent, 3 per cent and 5 per cent CO_2 in air. However, statistically significant differences in ventilation became manifest only when the groups were breathing 5 per cent CO_2 in air. The changes in minute ventilation ($\dot{V}_E$), respiratory frequency (f) and tidal volume (V_T) characteristic for each patient while breathing a mixture of 5 per cent CO_2 in air are listed in Table I and illustrated in Figure 2. It is clear that the increase in $\dot{V}_E$ is considerably less in patients with emphysema and CO_2 retention than in the three other groups. Thus mean $\dot{V}_E$ while the patient was breathing a mixture of 5 per cent CO_2 in air exceeded $\dot{V}_E$ while breathing ambient air by 187 per cent in the control group, by 146 per cent in the group of emphysema without CO_2 retention and by 58 per cent in the group of emphysema with CO_2 retention. The data on patients of the fourth group were not averaged because of the hetero-

geneous nature of the disorders represented; however, each of the patients responded as did the normal subjects. The increase in $\dot{V}_E$ in all groups was due primarily to increase in tidal volume (V_T) without statistically significant increase in respiratory frequency (f).

In Figure 3 is further depicted an interesting distinction between the ventilatory responses of the two groups of patients with emphysema. In the emphysema patients with CO_2 retention (Fig. 3A), $\dot{V}_E$ while breathing the mixture of 5 per cent CO_2 in air ($F_{ICO_2} = .05$) is closely related to $\dot{V}_E$ during breathing of ambient air ($F_{ICO_2} = 0$). A similar plot for the emphysematous patients without CO_2 retention is illustrated in Figure 3B, to which the best visual line of Figure 3A has been transposed. It is apparent that in the latter group there is (1) considerable augmentation of $\dot{V}_E$ while breathing the mixture of 5 per cent CO_2 in air and (2) no relation between $\dot{V}_E$ when $F_{ICO_2} = 0$ and $\dot{V}_E$ when $F_{ICO_2} = .05$.

2. Gas exchange in the lungs and arterial blood composition while breathing ambient air and 5 per cent CO_2 in air. In Table II are listed the data concerning respiratory gas exchange and arterial blood gaseous composition during the ventilatory studies listed in Table I. In all groups the respiratory exchange ratio (R_E) decreased during CO_2 breathing. This reflects in large part the lack of sufficient time during the short period of CO_2 breathing to restore fully the dynamic equilibrium for CO_2 throughout the lungs, blood and tissue fluid and indicates some degree of retention of inspired CO_2 in blood and tissues. Under these conditions calculations of the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in the lungs do not exactly measure the corresponding exchanges in the tissues, resulting in a higher $\dot{V}_{O_2}$ and lower $\dot{V}_{CO_2}$ than actually obtains.

In normal subjects the arterial blood P_{CO_2} and pH probably can be taken to be a measure of the main chemical stimulus to the respiratory center. It is therefore of interest that, even though the mean changes in arterial blood P_{CO_2} and pH during 5 per cent CO_2 breathing were of the same magnitude in the control subjects and in the two groups of patients with emphysema, being respectively +8, +8 and +7 mm. Hg and −0.6, −0.6 and −0.4 pH units (Table II), the patients with emphysema and CO_2 retention had a consistently lower ventilatory response to the inspired CO_2 mixture. Further analysis, relating arterial blood P_{CO_2} to alveolar ventilation ($\dot{V}_A$) rather than to minute ventilation ($\dot{V}_E$), indicates that in the emphysematous patient with CO_2 retention the increase in $\dot{V}_A$ is generally less than in the other control subjects and patients. This is illustrated in Figure 4. Finally, no relationship could be

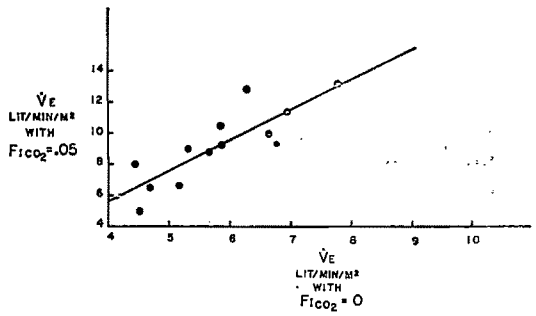


FIG. 3A. Correlation between minute ventilation while the patient is breathing ambient air and while breathing 5 per cent CO_2 in air in thirteen patients with pulmonary emphysema and CO_2 retention; the regression line is the best visual line through the points.

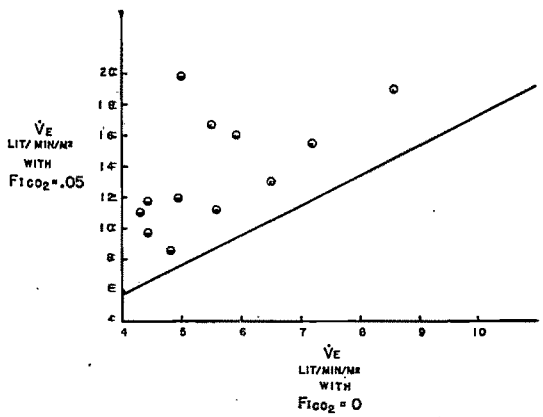


FIG. 3B. Correlation between minute ventilation while the patient is breathing ambient air and while breathing 5 per cent CO_2 in air in twelve patients with pulmonary emphysema without CO_2 retention. The visual line of Figure 3A has been included to facilitate comparison between the two groups of patients.

established between the level of alkali reserve (T_{40}) and the ventilatory response to 5 per cent CO_2 in air.

3. Effect of relief or aggravation of hypoxia on the ventilatory response to 5 per cent CO_2 in air. As a result of a limited increase in $\dot{V}_A$ while breathing 5 per cent CO_2 in air the patients with emphysema and CO_2 retention exhibited a slight rise in arterial blood oxyhemoglobin saturation (Table II) and oxygen tension (P_{aO_2}), the increments varying with the level of arterial oxyhemoglobin saturation prior to CO_2 breath-

TABLE II

GAS EXCHANGE AND ARTERIAL BLOOD COMPOSITION WHILE BREATHING AMBIENT AIR AND 5 PER CENT CO_2 IN AIR IN FOUR GROUPS

Subject	Breathing Mixture: Ambient Air						Breathing Mixture: 5% CO_2 in Air					
	Gas Exchange in Lungs			Arterial Blood			Gas Exchange in Lungs			Arterial Blood		
	$\dot{V}_{O_2}$* (L./min./M²)	$\dot{V}_{CO_2}$† (L./min./M²)	R_E‡	O_2 (sat. %)	P_{CO_2} (mm. Hg)	pH	$\dot{V}_{O_2}$ (L./min./M²)	$\dot{V}_{CO_2}$ (L./min./M²)	R_E	O_2 (sat. %)	P_{CO_2} (mm. Hg)	pH
Group I: Control Subjects												
E. Mc.	131	112	.86	94	37	7.43	144	96	.68	96	45	7.39
E. C.	138	109	.79	96	39	7.43	163	109	.67	99	41	7.41
A. O.	122	107	.88	98	35	7.42	140	96	.69	98	42	7.36
E. A.	127	113	.89	94	37	7.42	135	117	.80	99	47	7.35
J. A.	140	121	.86	94	35	7.47	145	121	.83	97	46	7.38
W. M.	143	126	.88	93	36	7.41	173	120	.70	98	45	7.33
A. F.	146	130	.89	96	44	7.43	125	101	.80	100	50	7.39
J. K.	146	144	.98	97	38	7.40	143	125	.87	96	46	7.35
W. S.	216	167	.77	95	37	7.43	226	170	.76	100	45	7.37
M. C.	148	113	.76	95	31	7.48	.....	.....	...	.....	....	....
B. L.	132	102	.78	95	33	7.42	.....	.....	...	.....	....	....
Average	144	122	.85	95	37	7.43	154	117	.76	98	45	7.37
Standard deviation	26.6	19.4	.05	1.4	3.2	.02	28.5	22.5	.02	1.4	2.5	.02
Group II: Patients with Chronic Pulmonary Emphysema without CO_2 Retention												
G. E.	130	107	.82	90	40	7.40	153	118	.77	96	43	7.38
G. P.	152	126	.83	90	40	7.39	157	109	.65	99	47	7.33
P. G.	110	93	.85	93	43	7.40	167	101	.60	95	56	7.34
J. H.	135	112	.83	95	43	7.40	139	105	.76	100	50	7.37
J. T.	136	122	.89	95	43	7.42	153	106	.69	100	49	7.38
L. T.	146	123	.84	95	39	7.46	174	114	.66	100	54	7.31
J. S.	131	117	.88	92	41	7.38	131	83	.64	99	52	7.29
T. Ba.	153	135	.89	96	36	7.41	152	124	.82	99	42	7.37
W. E.	156	133	.85	93	39	7.41	157	113	.73	95	45	7.37
J. C.	149	120	.79	94	36	7.43	160	108	.68	97	45	7.32
T. Br.	153	138	.90	95	40	7.42	139	108	.78	99	47	7.38
J. E.	151	123	.82	96	37	7.44	140	97	.70	100	47	7.36
Average	142	121	.85	94	40	7.41	153	107	.71	98	48	7.35
Standard deviation	13.1	15.7	.03	2.0	2.6	.02	12.7	10.1	.06	1.9	4.1	.03
Group III: Patients with Chronic Pulmonary Emphysema with CO_2 Retention												
A. P.	122	115	.95	83	50	7.43	125	87	.70	90	52	7.40
A. De.	120	112	.86	86	59	7.37	100	77	.78	92	66	7.35
A. Da.	138	124	.90	87	48	7.39	149	112	.75	94	58	7.31
J. Bu.	132	113	.86	85	50	7.43	184	113	.61	92	54	7.36
A. W.	158	147	.93	88	54	7.40	166	143	.87	91	60	7.36
A. Y.	110	100	.91	74	66	7.30	175	126	.72	84	70	7.30
W. B.	148	135	.91	86	58	7.39	140	115	.82	92	62	7.38
P. M.	157	128	.82	77	56	7.36	.....	...	...	93	60	7.32
B. B.	157	127	.81	88	52	7.39	154	112	.72	97	64	7.33
S. R.	152	140	.92	78	63	7.37	149	117	.79	91	68	7.35
F. P.	137	109	.80	82	47	7.39	164	97	.69	95	57	7.35
J. Be.	158	139	.88	93	46	7.40	166	127	.77	100	51	7.37
J. S.	140	132	.95	85	59	7.36	143	103	.72	92	66	7.27
Average	141	125	.88	84	54	7.38	151	111	.75	93	61	7.34
Standard deviation	12.4	13.5	.04	1.6	6.1	.03	21.9	15.5	.06	3.6	5.9	.03
Group IV: Miscellaneous Patients												
W. W.	117	99	.86	96	36	7.43	130	95	.73	100	43	7.36
C. S.	158	122	.78	94	38	7.49	166	121	.74	95	48	7.43
B. B.	150	127	.85	92	37	7.43	164	116	.71	95	51	7.37
N. P.	138	128	.92	91	35	7.42	169	118	.70	95	39	7.38
G. L.	166	139	.79	94	37	7.44	179	118	.66	82	43	7.33
E. H.	158	126	.80	3	41	7.40	206	128	.62	99	50	7.33

* $\dot{V}_{O_2}$ = oxygen uptake S.T.P.D.

† $\dot{V}_{CO_2}$ = carbon dioxide output S.T.P.D.

‡ R_E = respiratory exchange ratio in the lungs.

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ing. It was therefore necessary to estimate the contribution of a reduction in the hypoxic stimulus to the diminished ventilatory response to inspired CO_2 in these patients. Two series of experiments were devised.

a. Fourteen emphysematous patients with

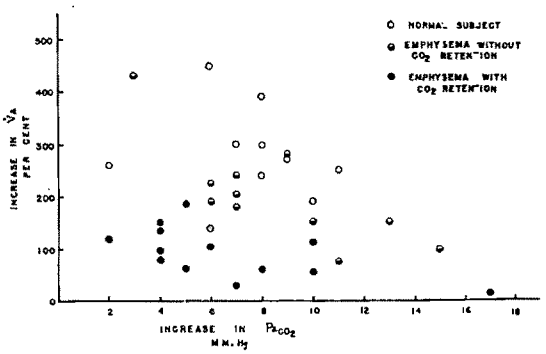


FIG. 4. Correlation between the increase in arterial blood CO_2 tension and the increase in alveolar ventilation in the three groups of subjects while breathing 5 per cent CO_2 in air; for discussion see text.

CO_2 retention and arterial oxyhemoglobin unsaturation were allowed to breathe a mixture of either 25 per cent or of 16 per cent O_2 in N_2. While breathing the 25 per cent O_2 mixture, mean P_{aO_2} increased from 48 to 65 mm. Hg but there was no associated change in $\dot{V}_E$; conversely, with the 16 per cent O_2 mixture a decrease in mean P_{aO_2} from 62 to 38 mm. Hg effected only a slight increase in $\dot{V}_E$. (Table III.)

TABLE III

EFFECTS OF CHANGES IN ARTERIAL BLOOD OXYGEN TENSION ON MINUTE VENTILATION IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA AND CO_2 RETENTION

F_{IO_2}*	No. of Studies	Average Arterial (% HbO_2)	Average P_{aO_2}† (mm. Hg)	Average P_{aCO_2}‡ (mm. Hg)	$\dot{V}_E$§ (L./min./M^2)
.21	8	79	48	55	5.5
.25	8	89	65	55	5.5
.21	6	91	62	50	8.6
.16	6	72	38	49	9.2

* F_{IO_2} = oxygen concentration in inspired gas.
† P_{aO_2} = oxygen partial pressure in arterial blood.
‡ P_{aCO_2} = carbon dioxide partial pressure in arterial blood.
§ $\dot{V}_E$ = minute ventilation B.T.P.S.

b. The patients of the three groups breathed successively (1) ambient air, (2) a mixture of 5 per cent CO_2 in air and (3) a mixture of 5 per cent CO_2 and 14 per cent O_2 in N_2, or a mixture of 5 per cent CO_2 in 95 per cent O_2. The changes in arterial blood P_{CO_2} while breathing the various 5 per cent CO_2 mixtures were similar in all three groups. (Table IV.) It is apparent that while breathing 5 per cent CO_2 the role of the moderate hypoxic stimulus in the control group and in the group with emphysema and CO_2 retention is negligible. However, when 5 per cent CO_2 was administered in 95 per cent O_2 a significant reduction in $\dot{V}_E$ occurred only

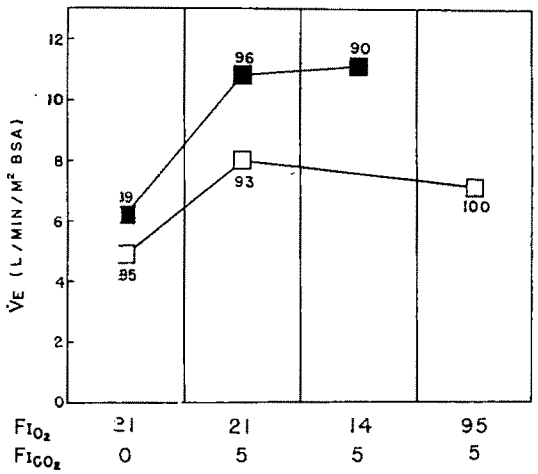


FIG. 5. Schematic representation of the average effect of breathing 5 per cent CO_2 with various mixtures of O_2 upon the minute ventilation in two series of observations on patients with chronic pulmonary emphysema and CO_2 retention. The figures next to the symbols represent the arterial blood O_2 saturation obtained with the various mixtures of inspired gas; each solid block represents the average of seven observations; each open block represents the average of five observations.

in the patients with emphysema and CO_2 retention. The average results of these studies in the patients with emphysema and CO_2 retention are illustrated in Figure 5.

In summary, a moderate relief of the hypoxic stimulus cannot account for the diminished ventilatory response characteristically observed in emphysematous patients with CO_2 retention during CO_2 breathing. On the other hand, increase in arterial blood P_{O_2} to levels far in excess of the physiologic range is capable of causing a marked diminution in $\dot{V}_E$. Such levels may result from pure oxygen breathing but are certainly not attainable while breathing 5 per cent CO_2 in air.

4. Effect of diminished ventilatory capacity on the ventilatory response to breathing 5 per cent CO_2 in air and to exercise. In each of the patients with emphysema and CO_2 retention the voluntary maximum breathing capacity (MBC) was considerably reduced. In six patients of this group the average increase in $\dot{V}_E$ while breathing the mixture of 5 per cent CO_2 in air was compared to the increase in $\dot{V}_E$ during a

standard exercise test and to the MBC. (Table v.) The data show that the $\dot{V}_E$ while the patient was breathing 5 per cent CO_2 increased only slightly above the control level and failed to reach a level higher than one-third of the MBC; by way of contrast, the $\dot{V}_E$ during mild exercise but also during more prolonged periods of steady state exercise, the results of which are not tabulated. On the other hand, in some patients with emphysema without CO_2 retention who showed a considerable reduction in MBC, $\dot{V}_E$ while the patient was breathing 5 per cent CO_2

TABLE IV

COMBINED EFFECTS OF VARIATIONS IN ARTERIAL BLOOD O_2 SATURATION AND CARBON DIOXIDE PARTIAL PRESSURE UPON VENTILATORY DRIVE IN THREE GROUPS

Inspired Gas Mixture	Group I: Control				Group II: Emphysema without CO_2 Retention				Group III: Emphysema with CO_2 Retention			
	No. of Experiments	Arterial Blood		$\dot{V}_E$ (L./min./M²)	No. of Experiments	Arterial Blood		$\dot{V}_E$ (L./min./M²)	No. of Experiments	Arterial Blood		$\dot{V}_E$ (L./min./M²)
		O_2 (sat. %)	P_{CO_2}* (mm. Hg)			O_2 (sat. %)	P_{CO_2} (mm. Hg)			O_2 (sat. %)	P_{CO_2} (mm. Hg)	
Ambient air.	8	96	37	5.3	..	...	..	.....	5	89	49	6.23
5% CO_2 in air.....	8	98	44	14.7	..	...	..	.....	5	96	56	10.80
5% CO_2 in 14% O_2...	8	95	42	15.1	..	...	..	.....	5	90	54	11.10
Ambient air.	3	95	36	5.5	4	95	40	5.07	7	85	52	4.89
5% CO_2 in air.....	3	99	46	16.1	4	99	50	14.00	7	93	61	7.98
5% CO_2 in 95% O_2...	3	100	46	16.2	4	100	49	13.30	7	100	62	6.59

* P_{CO_2} = partial pressure of carbon dioxide.
† $\dot{V}_E$ = minute ventilation B.T.P.S.

exercise reached two-thirds of the MBC. This disparity in response to CO_2 and exercise stimuli was repeatedly demonstrated in these patients, not only during brief periods of exercise but also during more prolonged periods of steady state exercise, the results of which are not tabulated. On the other hand, in some patients with emphysema without CO_2 retention who showed a considerable reduction in MBC, $\dot{V}_E$ while the patient was breathing 5 per cent CO_2 reached the level of ventilation obtained during the MBC test. In patient P. G., for instance, the MBC was 17.0 liters per minute, whereas $\dot{V}_E$ during 5 per cent CO_2 breathing was 17.9 liters per minute. Therefore, in patients with emphysema without CO_2 retention the MBC may constitute the limit of increase in $\dot{V}_E$ during CO_2 breathing; in this group an apparent reduction in responsiveness of the respiratory center to the CO_2 stimulus would, in fact, be due to restricted function of the chest bellows.

TABLE V

VENTILATORY RESPONSE TO DIFFERENT STIMULI IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA AND CO_2 RETENTION

Subject	Maximum Voluntary Breathing Capacity (L./min.)	Minute Ventilation		
		Ambient Air* (L./min.)	5% CO_2 in Air* (L./min.)	Ambient Air† (L./min.)
A. P.	30.0	8.1	10.4	20.0
A. Da.	38.0	7.3	13.1	17.8
B. B.	26.0	9.7	17.3	21.0
W. B.	67.0	10.7	14.4	22.6
A. Y.	27.0	7.2	10.1	13.7
A. Da.	32.0	6.9	7.5	18.0
Average	37.0	8.3	12.1	19.0

* At rest.
† During one minute exercise.

5. The effect of change in alkali reserve on the sensitivity of the respiratory center. In preliminary trials with oral administration of large doses of ammonium chloride and sodium bicarbonate to normal subjects it was not possible to cause a sustained change in alkali reserve. On the other hand, in a prolonged study of the effect of daily administration of 500 mg. of the carbonic anhydrase inhibitor diamox to seven patients with emphysema and CO_2 retention it was possible to assay the role of a reduction in alkali reserve upon the ventilatory response to inspired CO_2. The results in five of these seven patients have been presented briefly elsewhere.[11b]

TABLE VI

EFFECTS OF BREATHING 5 PER CENT CO_2 IN AIR ON ARTERIAL BLOOD COMPOSITION AND MINUTE VENTILATION FOLLOWING REDUCTION IN THE ALKALI RESERVE (T_{40}), IN SEVEN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMA AND CO_2 RETENTION

Date	Therapy	T_{40} (vol. %)	Breathing Mixture: Ambient Air			Breathing Mixture: 5% CO_2 in Air			$\frac{\dot{V}_{E(2)} - \dot{V}_{E(1)}}{\dot{V}_{E(1)}} \times 100$
			Arterial Blood		$\dot{V}_{E(1)}$ (L./min.)	Arterial Blood		$\dot{V}_{E(2)}$ (L./min.)	
			P_{CO_2} (mm. Hg)	O_2 (sat. %)		P_{CO_2} (mm. Hg)	O_2 (sat. %)		
Subject 1, A. P.									
11/19/52	Control	61	50	83	8.12	52	90	10.40	61
11/26/52	Control	56	56	84	7.77	60	91	11.50	56
12/3/52	Diamox	48	47	83	8.40	58	89	9.94	48
12/11/52	Diamox	49	53	90	6.97	61	89	8.86	49
12/29/52	Diamox	52	43	87	9.08	56	89	11.20	52
1/22/53	Diamox	48	46	91	8.27	56	97	10.60	48
2/9/53	Diamox	49	40	96	9.22	50	97	13.70	49
3/11/53	Diamox	49	43	94	9.96	50	100	13.50	49
4/29/53	Diamox	43	43	91	8.15	54	95	12.00	43
9/15/53	Diamox	47	43	81	8.35	52	90	11.00	47
11/17/53	Diamox	45	45	87	8.60	54	100	12.70	45
1/13/54	Control	55	54	73	9.95	61	92	13.80	55
Subject 2, A. W.									
12/22/52	Control	60	54	88	11.50	60	91	19.60	70
12/24/52	Control	..	..	..	11.70	..	...	15.70	34
12/31/52	Diamox	53	45	87	10.50	56	92	16.30	55
1/15/53	Diamox	53	48	88	11.00	60	94	16.10	46
1/21/53	Diamox	50	48	87	12.10	59	94	17.60	46
2/19/53	Diamox	52	57	84	9.38	64	94	13.80	47
3/5/53	Diamox	52	46	87	11.60	52	97	14.80	28
3/9/53	Diamox	50	47	88	12.20	56	93	16.00	31
4/27/53	Diamox	49	43	93	12.70	52	94	17.00	34
10/6/53	Diamox	52	54	87	9.57	63	94	13.50	41
Subject 3, A. Y.									
1/7/53	Control	56	66	74	7.25	70	84	10.10	40
2/19/53	Control	57	54	60	6.54	..	...	9.05	38
6/17/53	Diamox	48	48	84	6.70	57	95	9.72	45
11/24/53	Diamox	48	55	78	5.19	70	85	8.20	57
12/16/53	Diamox	47	51	85	6.12	59	94	8.39	37
1/31/55	Diamox	49	53	80	5.75	60	88	7.30	27
Subject 4, P. M.									
3/27/53	Control	59	54	68	11.30	..	...	16.90	50
4/8/53	Diamox	52	56	77	11.50	60	93	14.30	25
5/8/53	Diamox	49	43	74	12.20	53	85	17.60	44
5/18/53	Diamox	..	..	..	11.40	..	...	17.40	53
Subject 5, B. B.									
11/2/53	Control	53	52	88	9.74	64	97	17.30	77
1/19/54	Diamox	46	47	89	9.20	58	95	15.60	69
2/11/55	Diamox	41	39	93	8.97	50	98	10.97	22
Subject 6, W. B.									
1/26/53	Control	59	58	86	10.70	62	92	14.40	35
2/4/53	Control	55	50	87	11.70	58	94	13.30	12
2/20/53	Diamox	48	50	90	10.00	58	96	14.50	45
3/16/53	Diamox	47	46	91	12.40	50	95	19.00	53
4/8/53	Diamox	47	47	94	9.10	60	99	16.70	83
5/27/53	Diamox	48	45	94	8.12	54	97	14.10	74
Subject 7, A. De.									
11/21/52	Control	54	59	85	6.92	66	92	7.50	8
11/24/52	Control	55	54	87	6.72	60	96	9.20	37
5/21/53	Diamox	46	60	86	6.38	70	96	9.20	39
9/16/53	Diamox	54	56	88	5.17	62	92	9.46	83
11/25/53	Diamox	46	49	85	5.72	54	97	10.40	82
12/15/53	Diamox	48	53	86	5.26	59	96	8.37	59
2/10/55	Diamox	51	48	83	6.50	53	95	9.50	46

Serial measurements presented in Table VI for these seven patients indicate that there was, in all instances, a sustained reduction in alkali reserve to normal or nearly normal levels; there was also a less consistent reduction in arterial blood P_{CO_2} and a variable increase in arterial

year prior to diamox therapy. It is clear that arterial blood P_{CO_2} and T_{40} decreased during treatment with diamox. However, levels of the P_{CO_2} in arterial blood fluctuated somewhat during treatment. It is therefore pertinent to note that the arterial blood samples were drawn

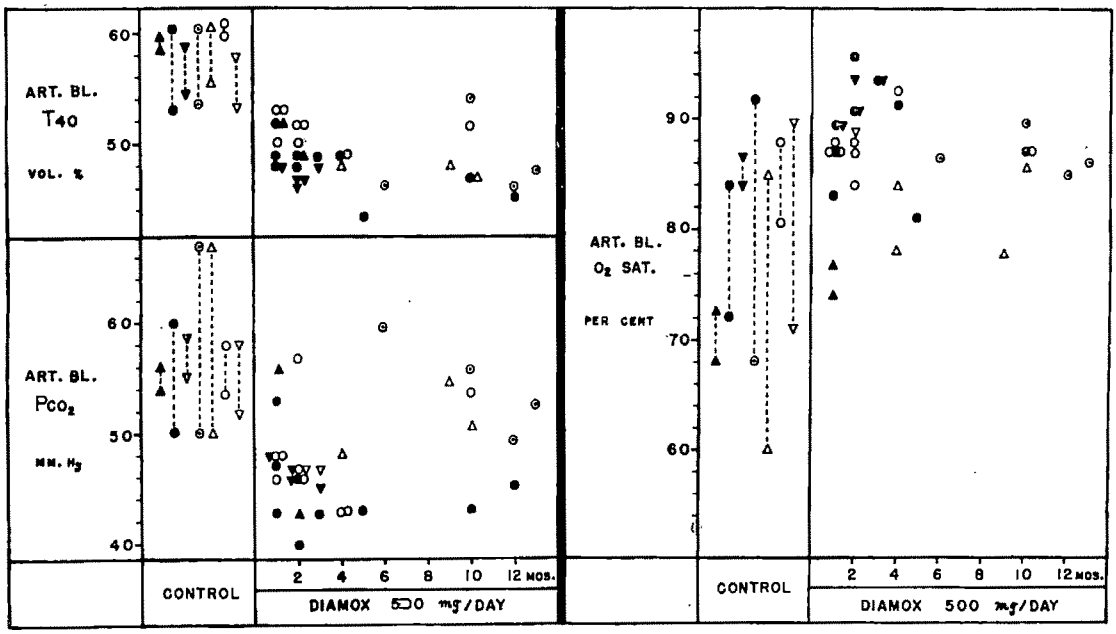


FIG. 6. Chart of the effect of diamox therapy upon the arterial blood alkali reserve, CO_2 tension and oxyhemoglobin saturation in seven patients with pulmonary emphysema and CO_2 retention. In the control box the range of variation during the year preceding diamox therapy is indicated by broken lines connecting the separate symbols designating each patient.

blood oxyhemoglobin saturation. Statistical analysis of the data collected prior to and during diamox therapy in this small group of patients showed (1) a decrease in mean arterial blood T_{40} from 57 vol. per cent (S.D. $\pm$ 2.8 vol. per cent) during the control period to 49 vol. per cent (S.D. $\pm$ 2.8 vol. per cent) during treatment, (2) a decrease in mean arterial blood P_{CO_2} from 55 mm. Hg (S.D. $\pm$ 4.4 mm. Hg) to 48 mm. Hg (S.D. $\pm$ 5.1 mm. Hg) and (3) an increase in mean arterial blood oxyhemoglobin saturation from 80 per cent (S.D. $\pm$ 9.3 per cent) to 87 per cent (S.D. $\pm$ 5.1 per cent) All these differences are statistically significant for $P < .01$.

In Figure 6 are plotted the consecutive changes in the alkali reserve (T_{40}), arterial blood P_{CO_2} and oxyhemoglobin saturation during the first year of diamox therapy. In this illustration the data of Table VI have been supplemented by inclusion of the range of arterial blood values for each patient during the

for analysis at varying time intervals after the daily dose of diamox, although usually they were drawn twenty-four hours later. Consequently it is to be anticipated that blood samples drawn within a few hours after ingestion of diamox and at the height of metabolic acidosis would have the highest P_{CO_2}; conversely, blood samples drawn twenty-four hours after a given dose of diamox, when arterial blood pH had returned virtually to normal levels, would have the lowest P_{CO_2}. The fluctuations in arterial blood P_{CO_2}, which have been observed by others[11c,11d] when the drug was given in divided doses, may therefore in fact be due to the sampling of blood at a time when the acute metabolic acidosis caused by diamox is still at its height. Despite these qualifications, the trend to reduction in arterial blood P_{CO_2} is unmistakable.

In five of the seven patients the increase in $\dot{V}_E$ while breathing 5 per cent CO_2 in air remained during diamox therapy within the pretreatment range. In only two of the patients

was the increase in $\dot{V}_E$ somewhat greater during diamox therapy than before. However, even in these two patients the increase in $\dot{V}_E$ remained substantially less than in either the control subjects or the patients with emphysema without CO_2 retention. A diagram illustrating the trend

management of CO_2 retention occurring in the course of chronic pulmonary emphysema.

I. Factors Regulating Ventilation

The generally accepted concept of the function of the normal respiratory center portrays a

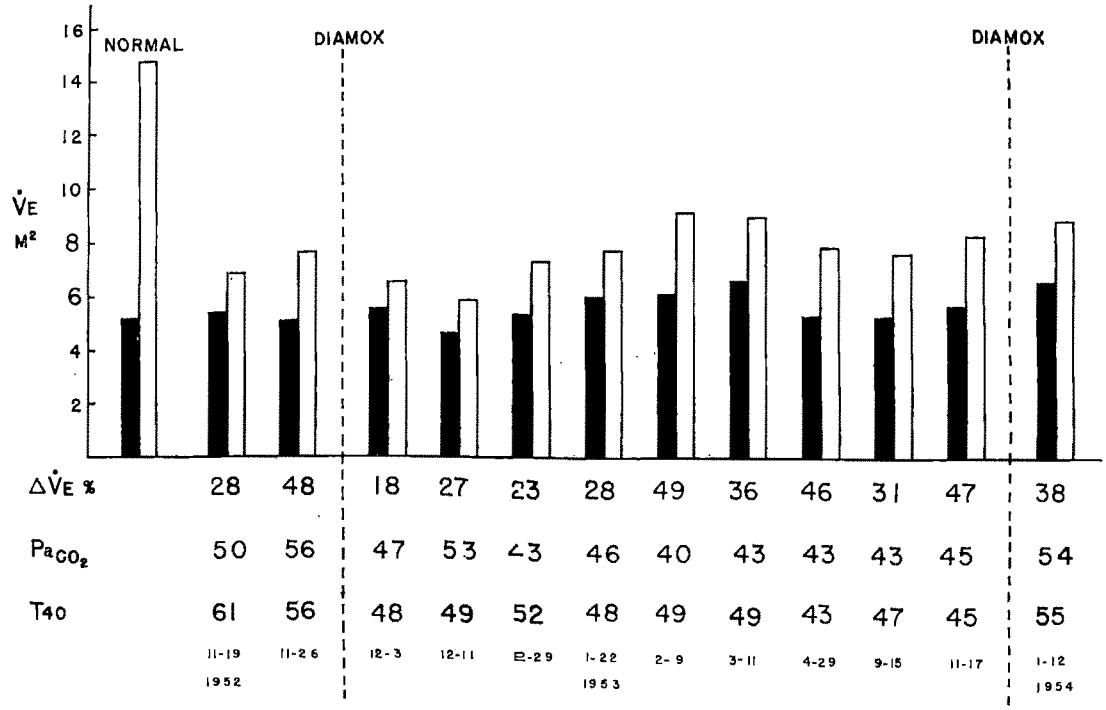


FIG. 7. Schematic representation of the influence of the arterial blood P_{CO_2} and alkali reserve upon the ventilatory response in patient A. P. before, during and after diamox therapy. Solid block represents the minute ventilation while breathing ambient air; open block represents the minute ventilation while breathing 5 per cent CO_2 in air; data obtained during diamox therapy are within the two broken vertical lines.

of changes usually observed during the prolonged periods of observation is presented for patient A. P. in Figure 7.

A statistical analysis of the increments in $\dot{V}_E$ while breathing the 5 per cent CO_2 mixture in these seven patients indicates a mean increase of 46 per cent (S.D. $\pm$ 20.2 per cent) prior to diamox therapy and 48 per cent (S.D. $\pm$ 15.3 per cent) during treatment. The difference between these values is not statistically significant for $P < .1$.

COMMENTS

The results of these studies may be considered from two points of view: (1) their contribution to the understanding of the factors involved in the regulation of ventilation and (2) their application to the development of a rational therapeutic program for the prevention and

stream of stimuli from diverse sources converging upon this center to be integrated into the efferent motor responses which constitute the normal ventilatory drive. These stimuli arise primarily (1) from the variations of carbon dioxide tension (P_{aCO_2}) or pH in the arterial blood acting directly upon the respiratory center, (2) from the variations of the oxygen tension in the arterial blood (P_{aO_2}) acting upon the chemoreceptors in the aortic and carotid bodies, (3) from the stretching of strategically located proprioceptors in the chest cage, the respiratory muscles, the lungs and the limbs and (4) from higher centers such as the cerebral cortex. In the normal resting subject an increase in arterial blood P_{CO_2} is the prime stimulus for augmentation of ventilation; by way of contrast, among patients with chronic pulmonary emphysema included in the present study is a group in

whom responsivesness to this stimulus is diminished. This study therefore makes possible an evaluation of other respiratory stimuli which are usually considered to play lesser roles in the regulation of ventilation.

A. Increase in Arterial Blood CO_2 Tension (P_{aCO_2}). It is beyond the scope of this paper to define the arterial blood P_{CO_2} stimulus as distinct from arterial or intracellular hydrogen ion concentration. However, under the conditions of these experiments the P_{aCO_2} must reflect, even if it does not quantitatively measure, the stimulus applied to specialized receptors in the cells of the respiratory center.

In the group of patients with chronic pulmonary emphysema and CO_2 retention, the sluggish response of the respiratory center to the P_{aCO_2} stimulus can be recognized by the slight increase in ventilation attending the artificial increase in P_{aCO_2} and by a resting minute ventilation, while breathing ambient air, which was lower than in the group of patients with emphysema without CO_2 retention. Indeed, in the group with CO_2 retention the minute ventilation while breathing ambient air is least in those patients in whom the ventilatory response to inspired CO_2 is most diminished.

The mechanisms underlying the diminished sensitivity of the respiratory center have not been uncovered by the present study; however, among the possible factors investigated three warrant further consideration: (1) Damage to the respiratory center during a period of particularly marked acute hypoxemia. Such acute episodes are often encountered in the course of the disease. It has been demonstrated in this and in other studies[12] that chronic hypoxemia, such as occurs in congenital heart disease, does not impair the ventilatory response to inspired CO_2. However, since nervous tissue is particularly susceptible to injury by severe hypoxemia, the possibility remains that acute episodes of bronchitis and bronchopneumonia may reduce arterial blood P_{O_2} to levels capable of causing permanent damage to some specialized cells of the respiratory center. (2) Prolonged hypercarbia. The extended stay of normal subjects in an environment enriched with CO_2 is known to impair the sensitivity of the respiratory center to the CO_2 stimulus[13] but sensitivity is restored after withdrawal from this noxious environment. However, since experiments in normal subjects have been concerned with exposure to ambient CO_2 limited to a few days, it cannot be stated whether or not more prolonged exposure would cause irreversible damage to the respiratory center. (3) Increase in alkali reserve. The level of the alkali reserve, as adjusted by the kidney and the body tissues in compensation for persistent alteration in arterial blood P_{CO_2}, may condition the ventilatory response to the CO_2 stimulus. Thus chronic reduction in alkali reserve, such as follows hyperventilation at altitude, is associated with an augmented sensitivity to P_{aCO_2}.[14,15] Furthermore, perfusion experiments in animals indicate that changes in the carbonic acid-bicarbonate ratio of cerebrospinal fluid may alter the ventilatory response.[8] Observations such as these constitute a basis for the suggestion that the impaired ventilatory response to inspired CO_2 in patients with pulmonary emphysema and carbon dioxide retention may be due to increase in alkali reserve.[4] However, in the present study reduction in alkali reserve resulting from long-term administration of the carbonic anhydrase inhibitor sulfacetamide (diamox) failed to demonstrate any consistent restoration of sensitivity of the respiratory center to inspired CO_2. The irreversibility of this depressed state will be discussed in greater detail subsequently.

B. Decrease in Arterial Blood O_2 Tension (P_{aO_2}). In normal subjects a decrease in arterial blood O_2 tension accomplished by reducing the O_2 content of inspired gas results in only a slight increase in minute ventilation ($\dot{V}_E$). The present study explored the possibility that, in the face of a reduction in the effectiveness of arterial blood P_{CO_2} as a ventilatory stimulus, a heightened sensitivity to a decrease in P_{aO_2} might exist; no evidence could, however, be adduced to support this premise. Indeed, patients with pulmonary emphysema and CO_2 retention respond as do normal subjects, since they experience little change in $\dot{V}_E$ in response to either aggravation of hypoxemia or restoration of P_{aO_2} to within physiologic range. However, as anticipated from earlier studies,[16,17] a considerable increase in P_{aO_2} and therefore of O_2 dissolved in plasma, such as occurs during breathing of 100 per cent O_2, elicits a marked decrease in $\dot{V}_E$.

In brief, within the range of variations in arterial blood P_{O_2} observed during ambient air breathing by both normal subjects and patients with pulmonary emphysema, the hypoxic stimulus appears to contribute little to the total ventilatory drive.

C. Proprioceptors in the Chest, Respiratory Muscles

and Lungs. A striking difference exists between the minute ventilation of patients with chronic pulmonary emphysema and CO_2 retention on the one hand and patients with diffuse pulmonary disease presenting the syndrome of alveolar-capillary block[18] on the other. In the latter group hyperventilation is considerable at rest, even though arterial hypoxemia may be absent and despite the presence of normal or reduced P_{aCO_2}. The initiation of this hyperventilation in the absence of adequate chemical stimuli has been ascribed to an excess of stretch stimuli initiated by the diffuse pulmonary disease; its persistence may then be due to an increase sensitivity of the respiratory center to arterial blood P_{CO_2} brought about by the reduction in alkali reserve which occurs as renal compensation for persistent hyperventilation. In sharp contrast, hyperventilation may not occur in patients with pulmonary emphysema and CO_2 retention as the disease progresses despite arterial hypoxemia and increased P_{aCO_2}. Obviously, proprioceptive reflexes arising from chest, respiratory muscles and lungs do not compensate for the diminished effectiveness of the arterial blood P_{CO_2} stimulus.

D. Proprioceptors in Limbs. It is well established that in normal subjects the increase in minute ventilation ($\dot{V}_E$) during exercise cannot be accounted for by the summation of known chemical stimuli.[19,20] The present study confirms the concept that during exercise there is a dissociation between limb stimuli and known chemical stimuli, since patients poorly responsive to an increase in arterial blood P_{CO_2} had, nonetheless, a marked increase in $\dot{V}_E$ during either a short standard exercise or a mild steady state exercise.

II. The Prevention and Control of CO_2 Retention

A. Noxious Effects of CO_2 Retention. The noxious influence of CO_2 retention on the evolution of chronic pulmonary emphysema may be considered under three headings: (1) physical, that is, the reciprocal effect on alveolar O_2 tension of an elevation of alveolar CO_2 tension; (2) physiologic, that is, the irreversible reduction in responsiveness of the respiratory center to one of its most potent stimuli and the renal compensation for respiratory acidosis, and (3) pharmacologic, that is, the action of increased CO_2 tension as a depressant of physiologic and mental processes, which occasionally progresses to the stage of narcosis and death.

1. *Physical:* It has been recently suggested[21] that the development of CO_2 retention during the course of chronic pulmonary emphysema might operate as a compensatory mechanism, teleologically directed at reducing the O_2 cost of ventilation, thereby increasing the amount of O_2 available for non-ventilatory work. Although this concept is intriguing in principle, it should be borne in mind that an elevation in alveolar P_{CO_2} while breathing CO_2-free air would, *per se*, cause a reduction in alveolar and arterial P_{O_2}, thereby requiring a series of compensating circulatory and tissue mechanisms in order to ensure adequate O_2 transport and utilization.

2. *Physiologic:* From these studies emerges the essential finding that the adverse physiologic effect of CO_2 retention upon the respiratory center, once established, is generally not reversible. A similar conclusion had previously been reached by indirect estimates of blood gas composition and shorter periods of clinical observation.[5] As a consequence, the automatic and adequate augmentation of alveolar ventilation, a prime compensatory mechanism for the disturbed alveolar ventilation-perfusion relationships which characterize pulmonary emphysema, is denied to the patient with this disease. However, the impaired ventilatory response to arterial blood P_{CO_2} is not the only factor limiting alveolar ventilation ($\dot{V}_A$); in planning an adequate therapeutic program it is obviously necessary to distinguish between a limitation in $\dot{V}_A$ due to this mechanism and that due to mechanical restriction of the chest bellows.

At this point it is perhaps worthwhile to stress that the problem of restoring the CO_2 content as well as the partial pressure of CO_2 to normal limits in these patients is of considerable magnitude. This difficulty stems from two facts: (1) The body tissue fluid supplements the circulating blood in constituting an enormous reservoir for CO_2, and (2) the regulation of the CO_2 content of this reservoir depends not only upon the lungs but also upon the action of the kidneys in controlling the reabsorption of bicarbonate and associated cations and the availability of tissue buffer base.[22] The interrelation between lung and kidney is emphasized in the demonstration that a high P_{aCO_2} such as may result from inadequate alveolar ventilation, is an essential stimulus leading to augmentation of the alkali reserve by the kidney.[22–24] The mechanism whereby the alkali reserve may be reduced during diamox therapy is quite clear; however, the

means by which arterial blood P_{CO_2} is concomitantly reduced is not as readily demonstrable. The most likely cause of reduction in the arterial blood P_{CO_2} and of increase in arterial blood oxyhemoglobin saturation is an improvement in alveolar ventilation. This mechanism has been predicated by others.[11c,11d] However, in the present study calculations of $\dot{V}_A$ from the O_2 uptake, respiratory exchange ratio and arterial blood P_{CO_2} before and after diamox therapy do not reveal a consistent increase. However, this result may be misleading since, according to the calculations employed, a drop in arterial blood P_{CO_2} of 5 mm. Hg (about twice the error of the technic) would result in an increase in calculated $\dot{V}_A$ of only approximately 10 per cent. Therefore it is possible that increases in $\dot{V}_A$, undetectable at the time of study, may have occurred. The mechanism whereby such an increase in $\dot{V}_A$ would be accomplished remains obscure, since it has not been possible to demonstrate increased responsiveness of the respiratory center to the CO_2 stimulus.

3. *Pharmacologic:* The observations of many investigators have established that acute exposure of normal subjects to increased CO_2 tensions may result in progressive depression of the central nervous system to the point of anesthesia. There are, furthermore, well documented observations which indicate that treatment of patients with pulmonary emphysema and CO_2 retention with high concentrations of O_2 in inspired gas may be followed by mental depression, somnolence and even coma. This sequence of events is generally ascribed to a diminution in alveolar ventilation and an increase in CO_2 retention incidental to suppression of the hypoxic stimulus. Less emphasis has been placed upon the clinical observation that the untreated patient with pulmonary emphysema and CO_2 retention is generally drowsy, apathetic and mentally sluggish. The relation of this state to chronic CO_2 retention is emphasized in the present study wherein a decrease in arterial blood P_{CO_2} and alkali reserve following prolonged diamox therapy has been shown to restore the mental alertness and vitality of these patients. Similar observations have been made by others during briefer periods of treatment with diamox.[25]

B. Methods for Prevention and Control of CO_2 Retention. Turning now to the therapeutic implications of these studies, two methods are currently available for the prevention and control of CO_2 retention, namely, the improvement of alveolar ventilation ($\dot{V}_A$), and the promotion of elimination of bicarbonate by the kidney.

1. *Improvement of alveolar ventilation* ($\dot{V}_A$): There are many ways to improve $\dot{V}_A$. These include breathing exercises, bronchodilators, reduction in pulmonary blood volume and substitution of mechanical for spontaneous regulation of ventilation.

2. *Promotion of bicarbonate excretion by the kidney:* The best method currently available is the use of carbonic anhydrase inhibitors which promote the excretion of bicarbonate and bicarbonate-bound base by the kidney, apparently by interfering with the hydrogen ion exchange mechanism for sodium.[22,23] These may also have some influence on CO_2 excretion but, although the amount of carbonic acid which may be excreted in the urine after diamox therapy is not established, it is probably insignificant. An additional effect of these inhibitors entails their role as weak diuretic agents which may indirectly augment $\dot{V}_A$ by decreasing the volume of blood in the lungs.

C. Clinical Applications of These Methods. The specific indications for the application of each of these methods depend upon the stage of the disease. From the point of view of therapy of CO_2 retention, patients with chronic pulmonary emphysema may be divided into three groups: (1) patients without CO_2 retention at rest or during activity, (2) patients with a tendency to CO_2 retention at rest or during exercise and (3) patients with chronic CO_2 retention. In all three groups acute bronchitis or pulmonary infection may precipitate or aggravate a state of respiratory acidosis. However, in the absence of acute respiratory infection the following general principles concerning the use of mechanical hyperventilators and diamox apply: (1) In the first group, mechanical hyperventilation and the use of diamox are not specifically indicated. (2) In the second group, attention should be directed toward the prevention of CO_2 retention. The use of mechanical ventilators for brief periods each day will promote the excretion of CO_2. In addition, patients addicted to O_2 therapy may find an adequate substitute in the increased alveolar O_2 tension provided by mechanical hyperventilation. The usefulness of diamox in these patients is not established. (3) In the third group, mechanical hyperventilation and diamox are indicated in an attempt to deplete CO_2 stores. In our experience with

patients of this group, previously studied over long periods of time, diamox has emerged as a valuable adjuvant to therapy. However, once initiated, its administration in one dose of 0.5 gm. per day has been continued indefinitely. Prolonged administration of diamox, with return of arterial blood P_{CO_2} and the alkali reserve toward normal, may occasionally obviate the need for mechanical hyperventilation.

Classification of a given patient according to the three groups indicated is based upon analysis of arterial blood for pH, total CO_2, P_{aCO_2} and alkali reserve, and responsiveness of the respiratory center to increase in P_{CO_2} in inspired gas. It is apparent that the most reliable guide to adequacy of any form of treatment is sequential analysis of arterial blood.

The distinction of these three groups of patients with chronic pulmonary emphysema may be obscured in acute bronchitis or bronchopneumonia which will bring about or aggravate elevation in arterial blood P_{CO_2}. However, a considerable elevation in the level of arterial blood P_{CO_2} and of alkali reserve in a patient who has not received O_2 therapy, a considerable degree of polycythemia or the presence of congestive heart failure suggest that the patient belongs to the third group of patients with chronic CO_2 retention rather than to the other two groups of patients with acute CO_2 retention. In any event the demonstration of respiratory acidosis by analysis of arterial blood demands the prompt institution of mechanical hyperventilation and diamox therapy to supplement the customary treatment with antibiotics, bronchodilators and, if need be, the specific measures for heart failure of the right side. Adequate mechanical hyperventilation is particularly essential if O_2 therapy is required to relieve severe arterial hypoxemia. It should be emphasized that occasional failure of diamox therapy is to be anticipated in patients with protracted and severe CO_2 retention and hypoxia[11d] if other therapeutic measures, particularly mechanical hyperventilation and O_2 therapy, are not instituted promptly, concomitantly and effectively. The value of diamox may be increased in the presence of congestive heart failure because of its mild diuretic action; in the patients with chronic pulmonary emphysema and CO_2 retention diamox therapy, apart from its diuretic action, facilitates clearing of the sensorium and reduces the hazard of CO_2 narcosis.

SUMMARY

1. The studies reported in this paper confirm the well documented observation that some patients with chronic pulmonary emphysema manifest an abnormally small increase in minute ventilation when CO_2 is added to inspired air. This diminished ventilatory response, despite a considerable increase in arterial P_{CO_2}, is characteristic of patients with chronic pulmonary emphysema who also have chronic CO_2 retention.

2. The mechanism underlying the abnormal ventilatory response in these patients appears to be a selective depression of the respiratory center to the stimulus of increased arterial blood P_{CO_2} and/or hydrogen ion concentration, since responsiveness to other stimuli such as exercise is well maintained. Restriction in the ventilatory capacity of the chest bellows is not the limiting factor to increase in minute ventilation during CO_2 breathing in patients with emphysema and CO_2 retention; by way of contrast, this restriction may suffice to limit the ventilatory response to the CO_2 stimulus in some patients with emphysema but without CO_2 retention.

3. The poor ventilatory response to the CO_2 stimulus is generally not reversible in patients with CO_2 retention of long duration even though the arterial blood P_{CO_2} and alkali reserve may be restored toward normal levels as a result of prolonged administration of the carbonic anhydrase inhibitor, diamox.

4. In addition to its action in decreasing the alkali reserve, diamox also reduces the arterial blood P_{CO_2}. The mechanism of this action is not established.

5. The relief or aggravation of hypoxemia within the physiologic range has little effect on the ventilatory response to inspired CO_2 in patients with emphysema and CO_2 retention. This suggests that the hypoxic stimulus is rather weak; however, as is well known, breathing pure O_2 consistently depresses the ventilatory response. The contrast between these observations emphasizes the importance of distinguishing between the physiologic and pharmacologic levels of O_2 tension in the blood.

6. The therapeutic implications of these observations, in patients with chronic pulmonary emphysema, are threefold. First, a distinction must be made between the effect of reduced ventilatory drive, and considerable restriction in ventilatory capacity in limiting minute ventila-

tion. Second, attention must be focused on the means for prevention and prompt relief of CO_2 retention. The role of diamox and of mechanical hyperventilation in the various stages of the disease has been described in this connection. Finally, by mobilizing large stores of CO_2 from the body, continuous diamox therapy in patients with prolonged CO_2 retention may restore mental alertness and a sense of well-being and diminish the threat of CO_2 narcosis.

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Pulmonary Arteriovenous Fistula*

Angiocardiographic Observations in Nine Cases

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PULMONARY arteriovenous fistulas are congenital hemangiomatous malformations of the pulmonary vascular bed. Recognition of the anomaly during life has been enhanced by the increased use of chest x-ray surveys, body section roentgenography, angiocardiography and safe exploratory thoracotomy. Sloan and Cooley[1] in 1953 reviewed eighty-five cases reported in the literature and added nine cases. In the short time since this review a total of over 150 cases[2–10] has been reported. The first clinical diagnosis of a pulmonary arteriovenous fistula was made in 1939 by Smith and Horton,[11] and the first successful surgical treatment was reported by Hepburn and Dauphiner[12] and performed by Shenstone and Janes[13] in 1942.

Pulmonary arteriovenous fistula rather than pulmonary arteriovenous aneurysm is the preferred name of the malformation and has become widely accepted. It calls to mind the characteristic features of the condition: afferent arterial and efferent venous connections. Furthermore, the term aneurysm usually refers to sac-like formations of dilated arterial vessels and does not take into account the venous component of the condition.

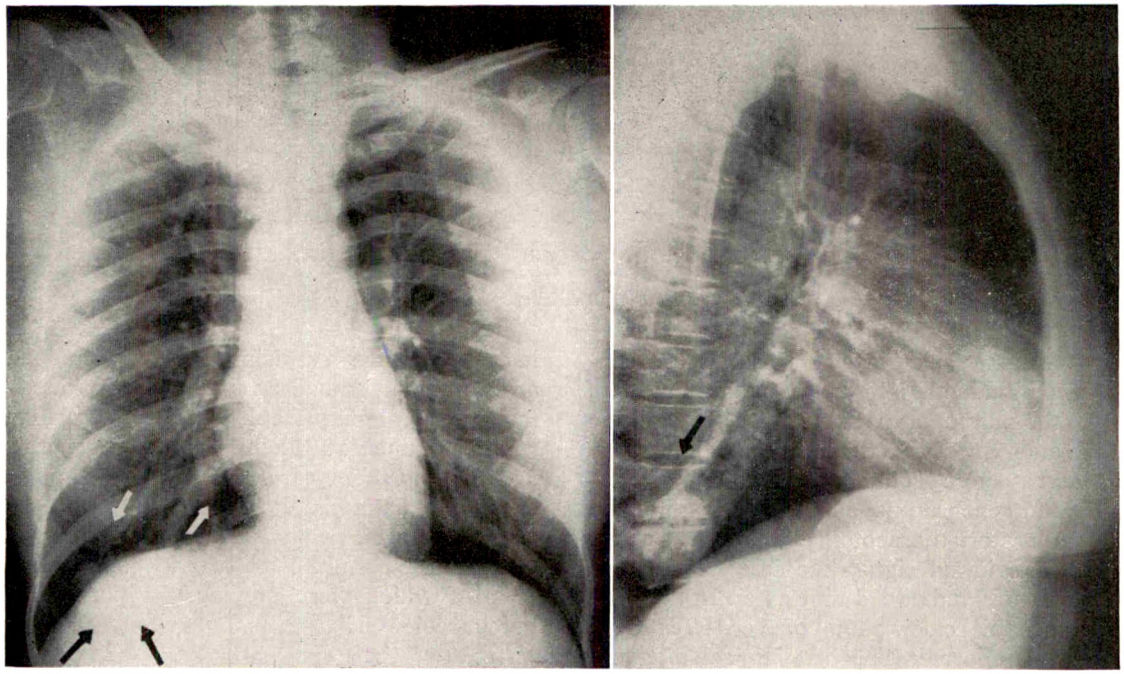
The diagnosis of pulmonary arteriovenous fistula formerly depended upon clinical findings such as cyanosis and clubbing of the fingers and toes, polycythemia and a murmur over a pulmonary lesion.[11–22] Later it was recognized that many patients with this clinical syndrome had associated cutaneous and mucosal hemangiomas and telangiectasis, and the hereditary nature of pulmonary arteriovenous fistulas and its relationship to Rendu-Osler-Weber's disease (hereditary capillary telangiectasis) became apparent.[16–24] Finally, with more experience came the realization that all the classical clinical features may be absent except for the roentgen findings.[23–25] Once suspicion of a pulmonary arteriovenous fistula is aroused, confirmation by fluoroscopy, laminography and angiocardiography will establish the diagnosis. It is generally agreed that angiocardiography provides the definitive diagnosis. For this reason the role that angiocardiography played in the diagnosis of nine cases of pulmonary arteriovenous fistulas (two of which were previously reported)[25] is herein reviewed.

CASE REPORTS

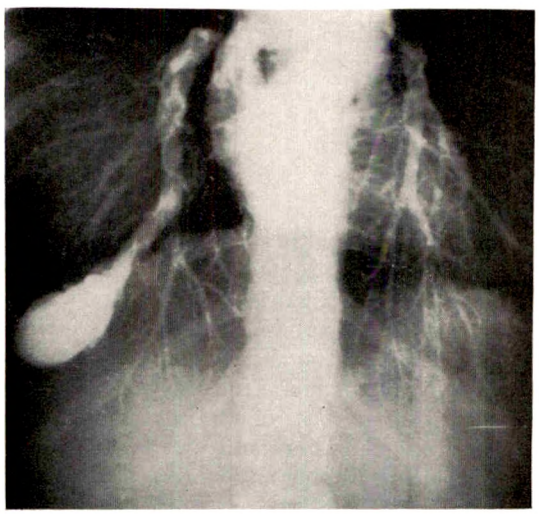
CASE 1. (Pulmonary arteriovenous fistula, posterior basilar segment, right lower lobe, associated with epistaxis, hemangioma of tongue and brain abscess). A thirty-nine year old housewife (New York Hosp., No. 596001) was admitted on March 22, 1950, because of right-sided convulsions and aphasia of three days' duration. There was a history of recurrent nosebleeds since childhood. On physical examination she was well developed and nourished but drowsy and aphasic with signs of right hemiplegia. A 6 mm. hemangioma was noted at the tip of the tongue. A harsh systolic murmur was heard at the base of the right lung which increased in intensity after deep inspiration. There was no clubbing or cyanosis of the fingers and toes.

Cerebral angiography and pneumoencephalography were normal but a left craniotomy disclosed a left parietal lobe abscess. The convulsions ceased but left hemiplegia and aphasia persisted. There were frequent nosebleeds during hospitalization. Chest x-rays (Fig. 1A and B) showed a heart of normal size and a rounded mass at the right base which had afferent and efferent vascular connections. Angiocardiography (Fig. 1C) confirmed the presence of an

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Aided by a grant from the Mallinckrodt Chemical Works.



1A 1B



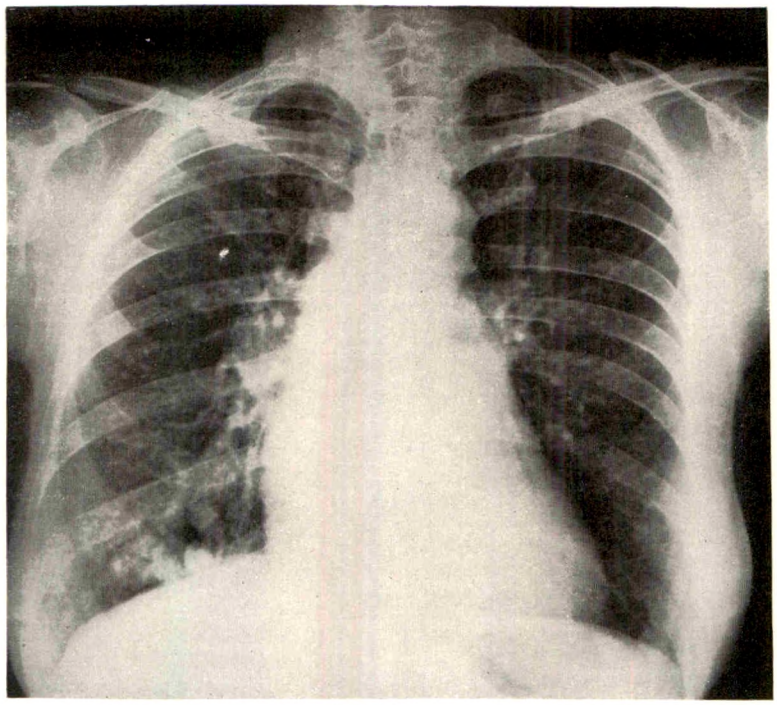
1C

FIG. 1. Case I. Pulmonary arteriovenous fistula, posterior basilar segment, right lower lobe complicated by brain abscess. A, conventional frontal roentgenogram showing a large single rounded density behind right diaphragm (arrows). Afferent (arrow) pulmonary arterial branch and efferent (arrow) venous communication readily discerned. B, right lateral conventional roentgenogram clearly reveals pulmonary arteriovenous fistula with characteristic afferent (arrow) and efferent communications. C, frontal serial angiocardiogram at four seconds after the beginning of injection shows the opacified arteriovenous fistula with its efferent communication proceeding towards the left hilum.

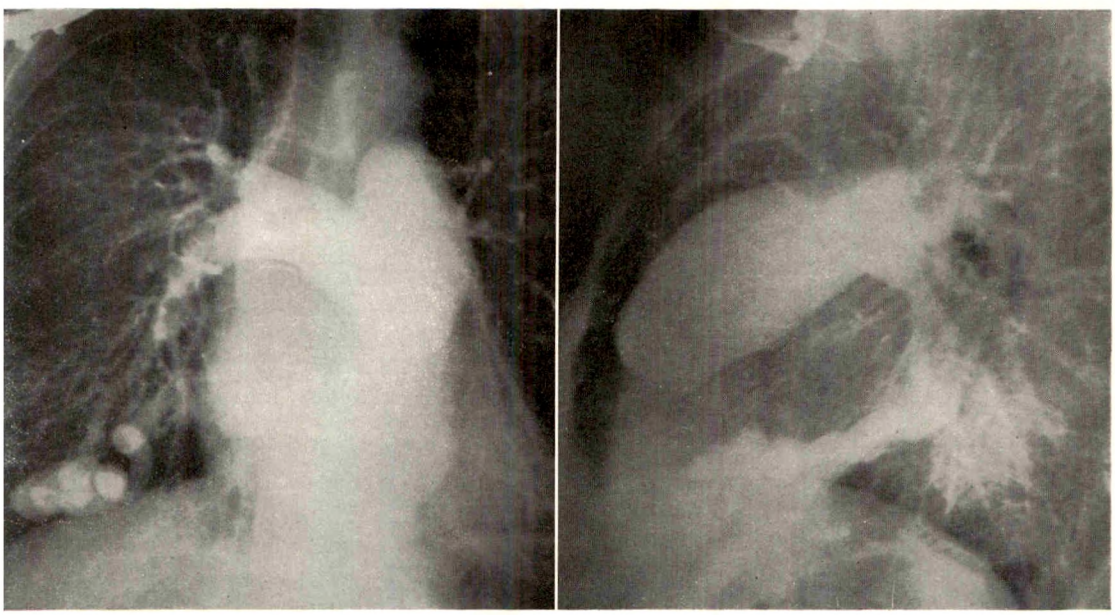
arteriovenous fistula in the posterior basilar segment of the right lower lobe.

The electrocardiogram was normal. On a return visit, July 22, 1954, examination disclosed persistence of the hemiplegia and aphasia but the patient was able to take care of her household chores. Her poor general condition precluded resection of the pulmonary arteriovenous fistula. Table I summarizes the findings.

CASE II. (Pulmonary arteriovenous fistula, medial basilar segment, right lower lobe, associated with rheumatic heart disease and hypertension.) A sixty-five year old housewife (New York Hosp., No. 319819) was first admitted in 1946 because of blurred vision. A middle fossa meningioma was found, and removal of the tumor resulted in improvement of vision. During her stay evidences of mitral stenosis and insufficiency were elicited. In 1947 she returned because of epigastric cramps. Urologic study at that time disclosed cysts of the right kidney, which were removed. Chest x-ray (Fig. 2A)

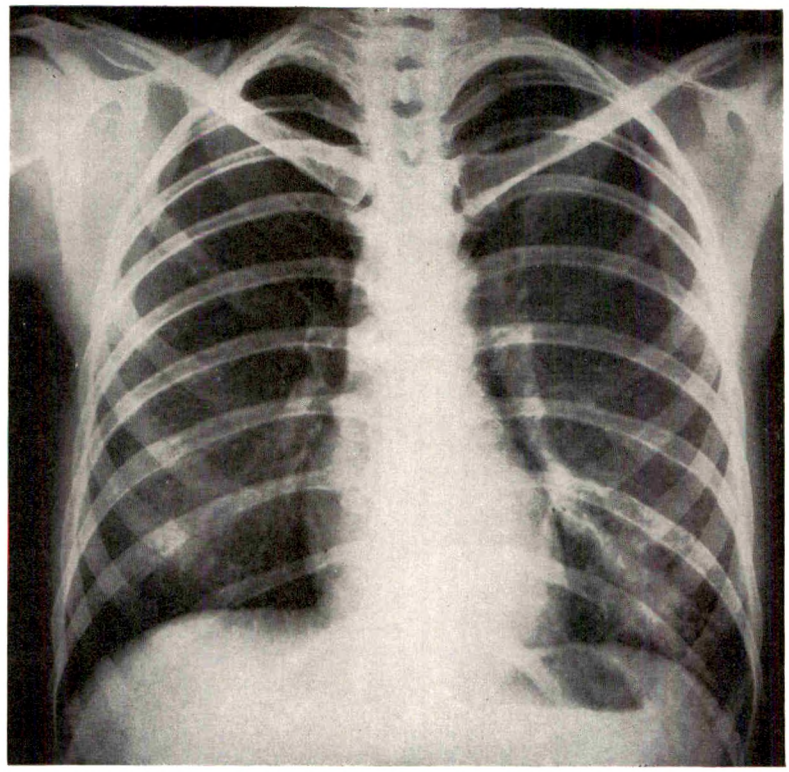


2A

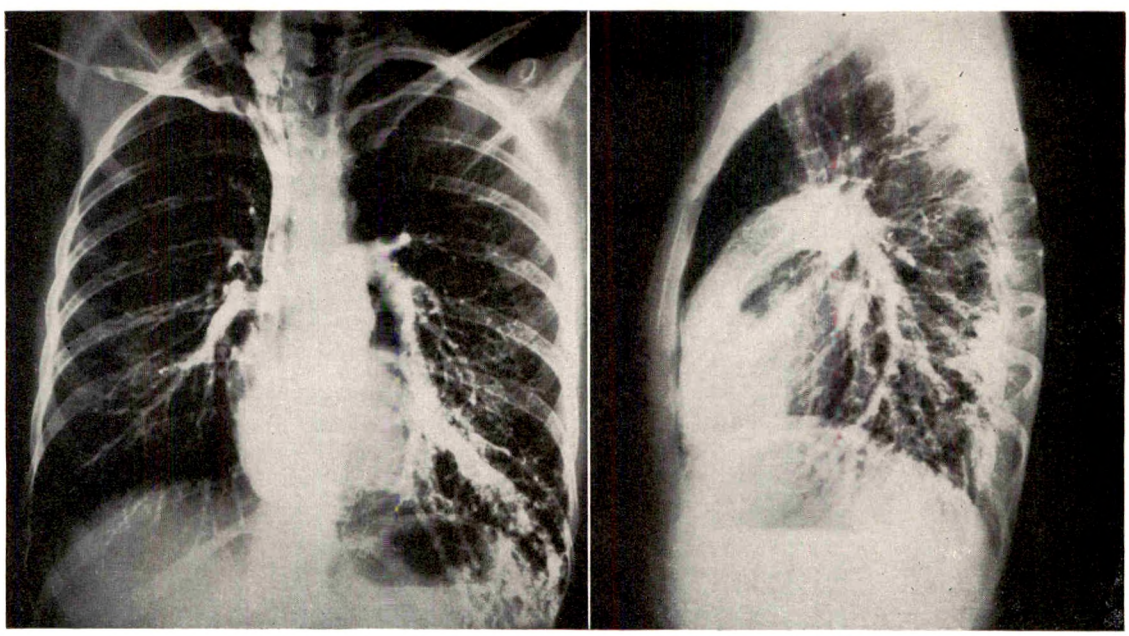


2B 2C

FIG. 2. Case II. Pulmonary arteriovenous fistula, medial basilar segment, right lower lobe in a patient with rheumatic heart disease (mitral stenosis). A, conventional frontal roentgenogram showing a nest of small nodular densities just above the right diaphragm. The heart, pulmonary artery and branches are enlarged. B, serial frontal angiocardiogram at five seconds shows a large pulmonary artery and branches. A large segmental pulmonary artery from the right descending branch of the pulmonary artery proceeds to the right lower lobe connecting with a coiled series of rounded densities (arteriovenous fistula) which in turn empty via an efferent vessel into the left atrium. C, left lateral angiocardiogram at three seconds shows the opacified main stem pulmonary artery and branches with an afferent basilar segmental division connecting the circinate arteriovenous fistula.

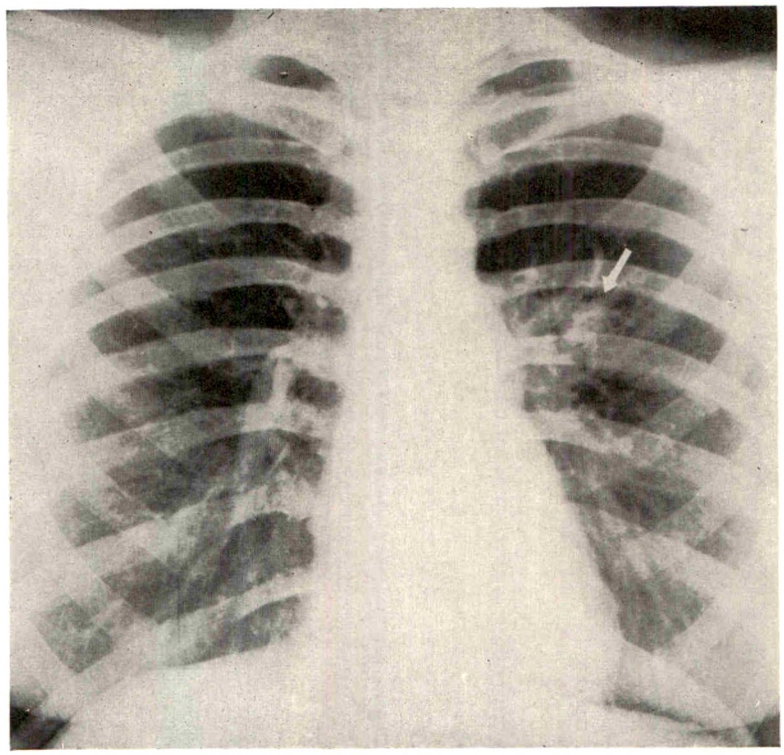


3A

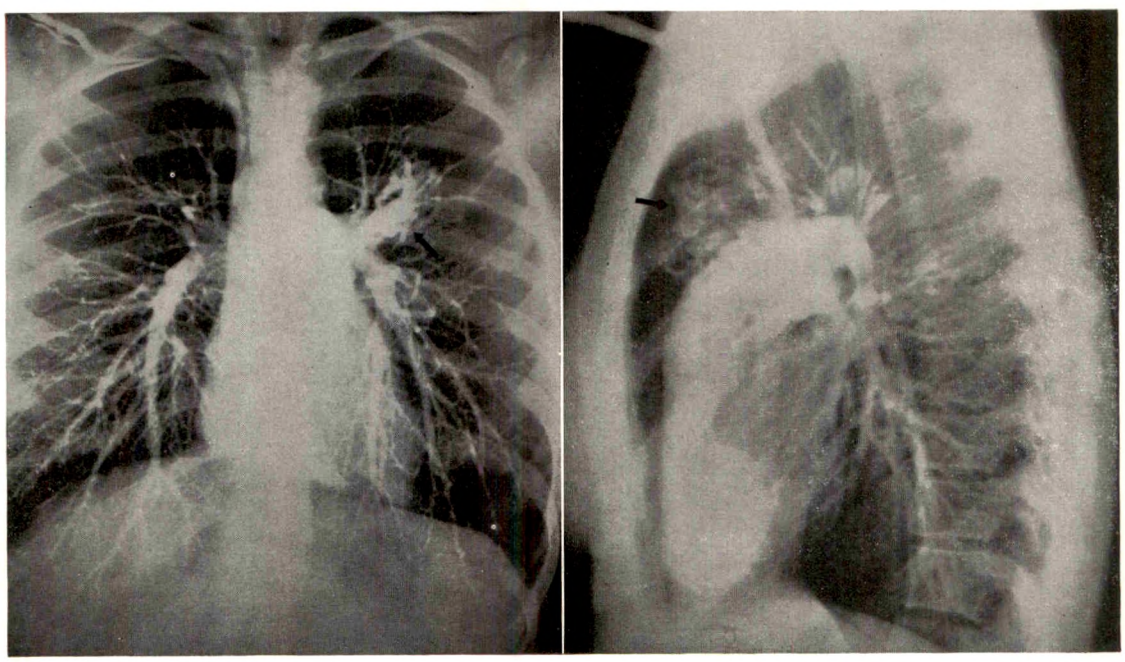


3B 3C

FIG. 3. Case III. Left lower lobe arteriovenous fistula in a thirty-one year old woman with cyanosis, polycythemia and clubbed fingers. A, conventional frontal roentgenogram showing linear hazy infiltrate-like density at left base. B, frontal angiocardiogram at two and one-half seconds showing the arteriovenous fistula. C, left lateral angiocardiogram at two and one-half seconds again demonstrating the left lobar arteriovenous fistula.[54]

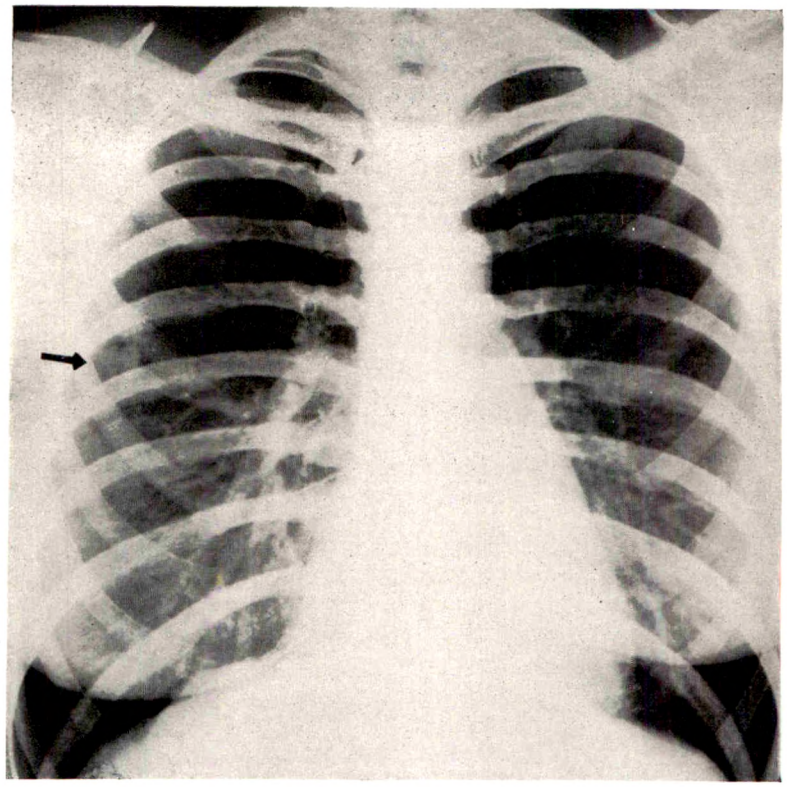


4A

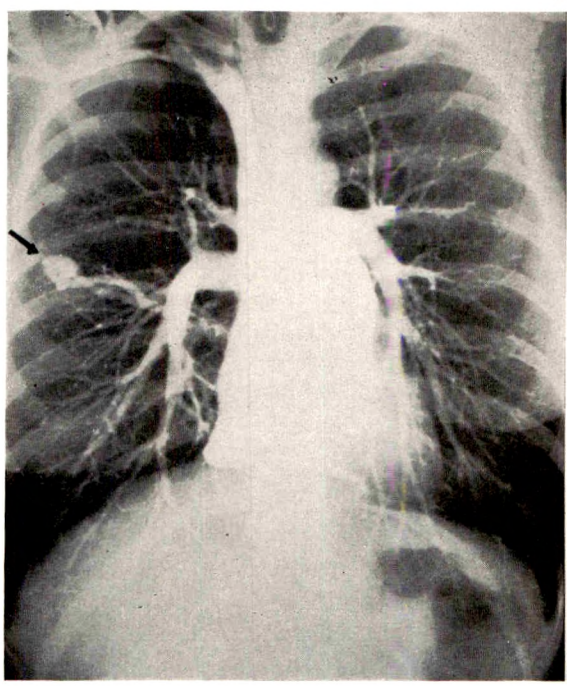


4B 4C

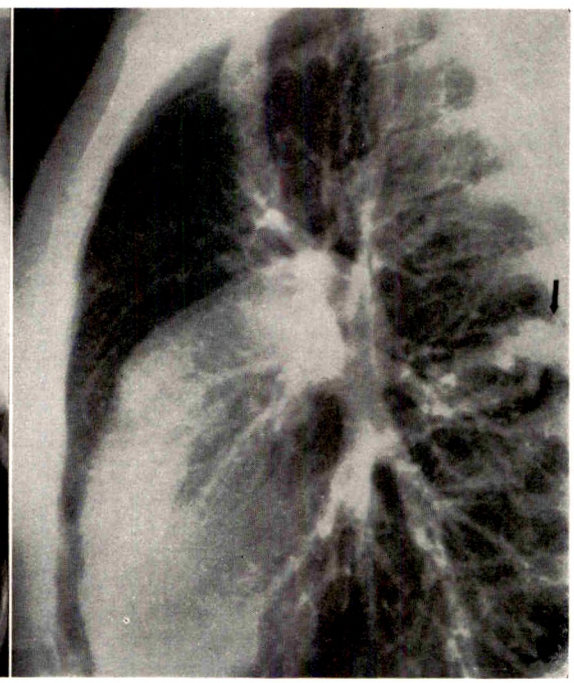
FIG. 4. Case IV. Anterior segment left upper lobe arteriovenous fistula in an asymptomatic sibling. A, conventional frontal roentgenogram reveals an "infiltrate" (arrow) left hilar region. B, frontal angiocardiogram at two and one-half seconds shows the arteriovenous fistula (arrow). C, left lateral angiocardiogram at two and one-half seconds localizes the arteriovenous fistula (arrow) in the anterior segment of the left upper lobe.[25]



5A



5B



5C

FIG. 5. Case v. Apical segment right lower lobe arteriovenous fistula in an asymptomatic sibling. A, frontal conventional roentgenogram reveals two tiny rounded densities (arrow) in outer portion right mid-lung field. B, frontal angiocardiogram at two and one-half seconds reveals the arteriovenous fistula with its opacified vascular connections. C, left lateral serial angiocardiogram shows the fistula in the apical segment of the right lower lobe.

revealed nodular densities at the right base. The heart was enlarged, especially the right ventricle, pulmonary artery segment and left atrium. The blood pressure in 1946 was normal but in 1947 it had increased to 220/110.

In 1949 cholelithiasis with obstructive jaundice developed which necessitated cholecystectomy and choledocholithotomy. Auricular flutter appeared prior to operation and was converted by digitalization to fibrillation. The postoperative period was stormy but the patient improved and was discharged. Subsequently she was seen periodically in the out-patient department. Early in 1951 central retinal artery thrombosis developed and she was readmitted to the hospital. This time the findings at the right base were suspected to be due to pulmonary arteriovenous fistula; however, no bruit was heard and clubbing and cyanosis of fingers and toes were not present. Angiocardiography (Fig. 2B and C) was performed and an arteriovenous fistula of the anterior basilar segment of the right lower lobe was demonstrated.

The electrocardiogram showed right axis deviation; the rhythm varied between auricular flutter and fibrillation. The patient was last seen on June 24, 1954. She was totally blind, but polycythemia, clubbing and cyanosis of the fingers had not developed. Because of her poor general health and the asymptomatic character of the pulmonary arteriovenous fistula, operation was not advised. (Table I.)

Case III. (Pulmonary arteriovenous fistula of the left lower lobe associated with polycythemia, cyanosis and clubbing of fingers and toes.) A thirty-one year old housewife (New York Hosp., No. 322967) had a routine prenatal chest film (Fig. 3A) at a New York City Health Department Chest Clinic, which disclosed an abnormal shadow at the left base. Following delivery dyspnea developed, and clubbing and cyanosis of the fingers and toes were noted. A bronchogram showed no evidence of bronchiectasis. Subsequently a vascular bruit was heard at the left base and the patient was referred for angiocardiography. On angiocardiography a left lower lobe arteriovenous fistula was demonstrated (Fig. 3B and C) and she entered the hospital for operation.

On admission April 19, 1950, the patient was found to be well developed but poorly nourished and chronically ill. Her heart was not enlarged; a blowing systolic murmur was heard at the pulmonic area. In the lower posterior

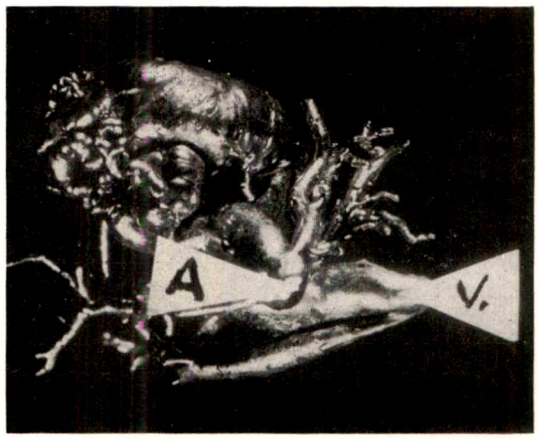


Fig. 5D. Case v. Apical segment right lower lobe arteriovenous fistula in an asymptomatic sibling; low melting alloy cast of specimen, A, afferent artery and V, efferent vein.[25]

area of the left lung, in the mid-axillary line, a loud bruit with systolic and diastolic components was heard. The murmur decreased after deep inspiration and disappeared after the Valsalva maneuver. The blood pressure was 105/75.

An electrocardiogram showed normal sinus rhythm at a rate of 72 per minute and no deviation of the electrical axis. Fluoroscopic examination failed to reveal vascular pulsations at the left base, or change in size of the left lower vascular tree with respiratory movements. Operation was advised but refused; the patient went home and has not returned for follow-up examination. (Table I.)

Case IV. (Asymptomatic pulmonary arteriovenous fistula, anterior segment, left upper lobe, in a sibling treated with lobectomy.) A nineteen year old typist (New York Hosp., No. 573907), previously reported on,[25] was found to have a pulmonary infiltrate (Fig. 4A) in 1950 when she applied for working papers. Because of a negative tuberculin test she was referred by a New York City Department of Health Tuberculosis Clinic for angiocardiography. This revealed (Fig. 4B and C) an arteriovenous fistula of the anterior segment of the left upper lobe. The patient was well developed and nourished, asymptomatic, and presented no abnormal physical findings. Subsequently a faint vascular bruit was heard, especially after deep inspiration, over the third left anterior interspace. A left upper lobectomy was performed and the patient made an uneventful recovery. (Table I.)

Case V. (Asymptomatic pulmonary arterio-

venous fistula, apical segment, right lower lobe, in a sibling treated with segmental resection.) A twenty-two year old housewife (New York Hosp., No. 616053), previously reported on,[25] had been aware of a right lung infiltrate following a chest x-ray survey at the age of fourteen years.

TABLE I

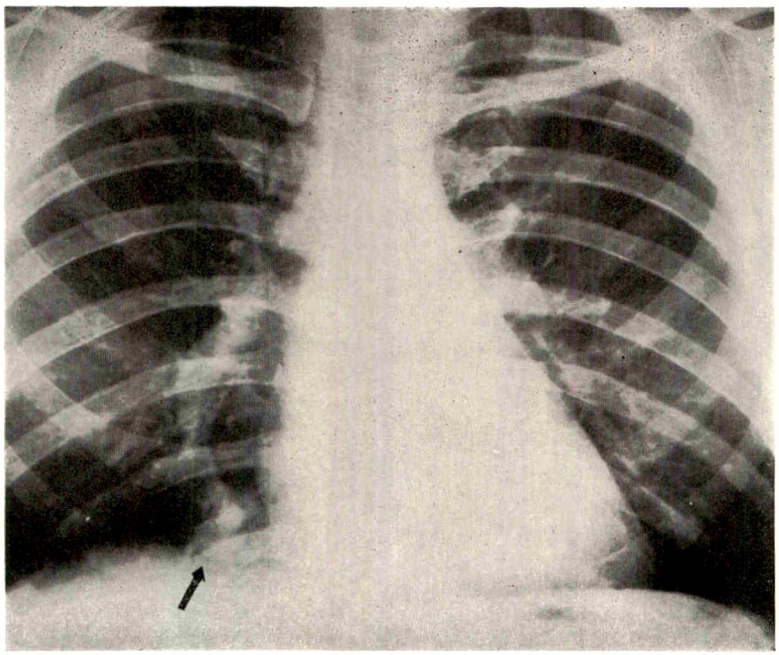
SUMMARY OF FINDINGS IN EIGHT CASES OF PULMONARY ARTERIOVENOUS FISTULAS

Case, Age, Sex	Lung	Lobe or Segment	Associated Conditions	Bruit	Hemoglobin (gm./100 cc.)	Red Blood Count (m./cu./mm.)	Hematocrit (%)	Comment, Operation and Follow-up
I, 39, F	R	Posterior basilar segment, right lower lobe	Epistaxis, hemangioma of tongue; left parietal lobe brain abscess	+	11.6 to 13.5	5.2	..	Convulsions ceased after drainage of brain abscess; hemiplegia persists; 4 yr. follow-up shows no change in cerebral or chest conditions; poor general condition precluded excision of fistula
II, 67, F	R	Medial segment, right lower lobe	Rheumatic heart disease and hypertension	0	13.5 to 16.4	4.7 to 5.6	..	During 4 yr. follow-up patient has had 3 operations (excision of meningioma, nephrectomy and cholecystectomy); auricular flutter and fibrillation and central retinal artery thrombosis occurred but lung lesion remains unchanged; operation of lung lesion not advised
III, 31, F	L	Left lower lobe	Dyspnea, polycythemia, cyanosis, clubbing of fingers	+	18.8	5.7	57	Operation refused; no follow-up
IV, 19, F	L	Anterior segment, left upper lobe	Asymptomatic	+	13.5	5.4	44	Sibling, remains asymptomatic after left upper lobectomy
V, 22, F	R	Apical segment, right lower lobe	Asymptomatic	+	14.5	4.1	42	Sibling, remains asymptomatic after segmental resection
VI, 54, M	R	Posterior basilar segment, right lower lobe	Subacute thyroiditis	+	14	4.7	48	Right lower lobectomy because of bronchial arterial connection of fistula; uneventful recovery ensued
VII, 31, M	R	Right middle lobe	Acute meningoencephalitis	+	15.6	4.9	..	Complete recovery from meningoencephalitis after 4 mo. antibiotic and chemotherapy; lobectomy, right middle lobe, no complications
VIII, 36, M	L	Lingular segment, left upper lobe	Asymptomatic	+	17	5.7	..	Abnormal shadow left lung discovered on routine chest film; lingulectomy and uneventful recovery
IX, 50, F	R L	Superior segment, right upper lobe Posterior basal segment, left lower lobe	Dyspnea, weakness, epistaxis, familial telangiectasis, cyanosis of finger tips	+ +	9.9	4.0	35	In spite of classical symptoms and signs of Rendu-Osler-Weber's disease and associated pulmonary arteriovenous fistulas, diagnosis only recently made; lobectomy left lower lobe; excision of fistula, posterior segment, right upper lobe planned

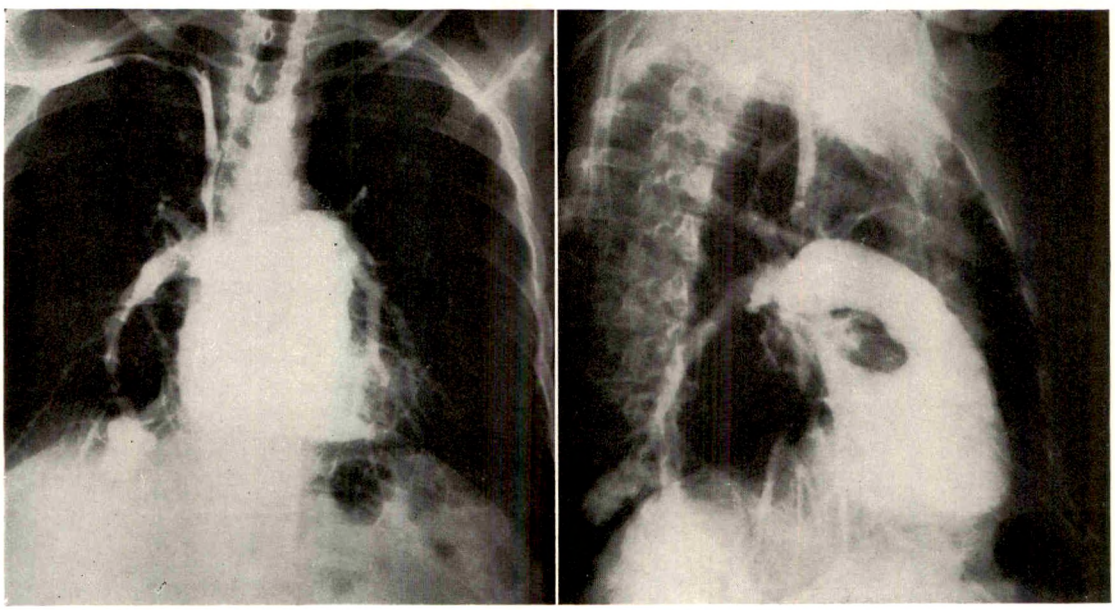
(Fig. 5A.) Following the finding of a pulmonary arteriovenous fistula in her sister (Case IV) she had an angiocardiographic study (Fig. 5B and C) which disclosed an arteriovenous fistula of the apical segment of the right lower lobe. She was asymptomatic. Physical examination revealed only a faint systolic murmur at the sixth interspace in the right mid-axilla.

On January 22, 1953, segmental resection of the apical segment of the right lower lobe was performed. The patient made an uneventful recovery. (Table I.) A low-melting alloy cast of the excised specimen (Fig. 5D) showed the single afferent artery (*A*) to be rather straight, slightly dilated and the source of a normal number of arterioles. It abruptly entered, through three branches, a single tortuous cluster of saccules almost 2 cm. in diameter which was drained by a single dilated vein (*V*) soon joined by another vein from adjacent lung.

CASE VI. (Asymptomatic pulmonary arteriovenous fistula, posterior basilar segment, right lower lobe, treated with lobectomy.) A fifty-four year old sales manager (New York Hosp.,



6A



6B 6C

FIG. 6. Case VI. Posterior basilar segment arteriovenous fistula of right lower lobe in a patient with subacute thyroiditis. A, conventional frontal roentgenogram reveals a rounded density above the right diaphragm (arrow). B, frontal angiocardiogram clearly shows the arteriovenous fistula with its afferent arterial and efferent venous connections. C, right anterior oblique angiocardiogram localizes the arteriovenous fistula in the right posterior basilar segment of the right lower lobe.

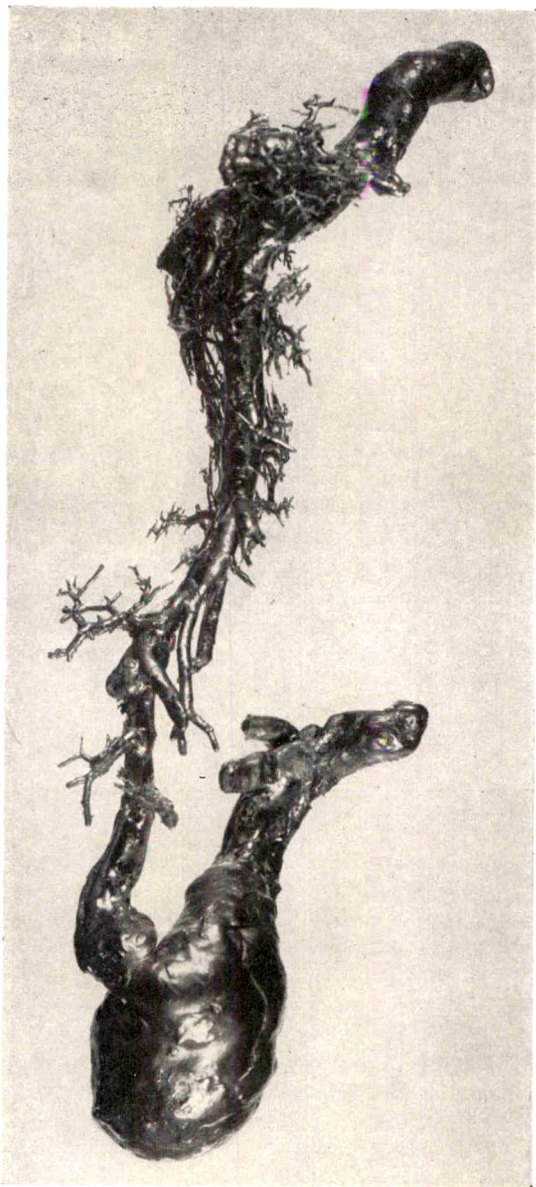


FIG. 6D. Case VI. Posterior basilar segment arteriovenous fistula of right lower lobe in a patient with subacute thyroiditis; low melting alloy cast of the fistula.

No. 678872) was admitted because of thyroid swelling which was diagnosed as subacute thyroiditis (Quervain's disease). A routine chest x-ray (Fig. 6A) disclosed a rounded nodular parenchymal shadow at the right base, closely related to the descending branches of the right pulmonary artery. At the lower pole of the lesion an efferent branch proceeding toward the right hilus was seen. Prior to admission this area had been interpreted as being due to pulmonary metastases or a hamartoma.

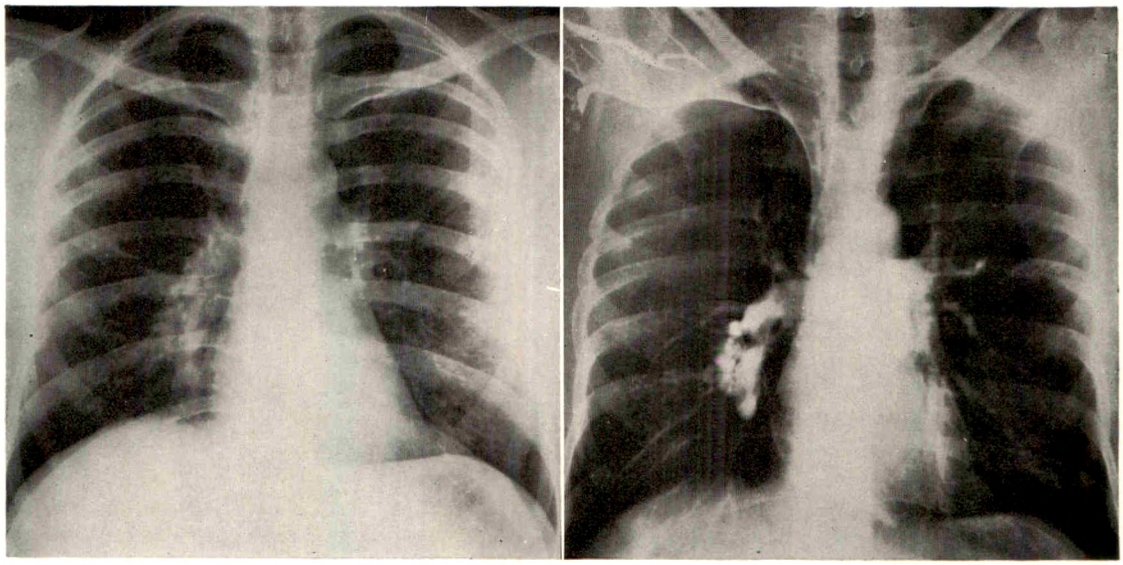
Angiocardiography (Fig. 6B and C) clearly established the presence of a pulmonary arteriovenous fistula of the posterior basilar segment of the right lower lobe. Previously, a vascular bruit was heard at the right base. Cyanosis and clubbing of the fingers and toes were not present. The heart was normal; the blood pressure was 130/80; the electrocardiogram was also normal.

At operation on May 26, 1954, three large lower lobe pulmonary vessels were seen. A ligature placed around the largest (middle branch) failed to collapse the pulsating arteriovenous fistula. Only after ligation and division of the pulmonary arteries and the bronchus of the lower lobe with its bronchial arterial blood supply (lobectomy) did pulsation of the fistula cease. The patient made an uneventful recovery. (Table I.)

The excised fistula was injected with low melting alloy with some difficulty as its main afferent vessel was found only after a long search. (Fig. 6D.) The artery followed a long, gently curved course to the posterior basal segment and then proceeded by a single branch into an extremely thin-walled sac with filmy septations. A single dilated vein drained the sinus.

CASE VII. (Pulmonary arteriovenous fistula, right middle lobe, treated with lobectomy; complicated by acute meningoencephalitis.) A thirty-one year old laborer (New York Hosp., No. 682240) was referred for angiocardiography from the Brooklyn Veterans Administration Hospital. The patient became acutely ill in November, 1953, with bitemporal headaches, episodes of unconsciousness, olfactory hallucinations and generalized convulsions. Fever, a generalized maculopapular rash, nuchal rigidity, hyperflexia, increase in spinal fluid cells and proteins with a diminished sugar content were present. Spinal and blood cultures were sterile. Complete recovery occurred after four months of intensive treatment with antibiotics and chemotherapy. The final diagnosis was acute meningoencephalitis.

While the patient was at the Veterans Hospital a grade II systolic murmur was heard over the right anterior chest at the fourth anterior interspace near the sternum. The electrocardiogram was normal. Chest roentgenogram (Fig. 7A) disclosed increased vascularity in the right hilus which on lateral view was located in the middle lobe. Angiocardiography



7A 7B

FIG. 7. Case VII. Right middle lobe arteriovenous fistula in a patient with meningoencephalitis. A, conventional frontal roentgenogram shows increased vascularity of right hilar vessels. B, frontal angiocardiogram shows arteriovenous fistulous malformation. Lateral view (not shown) showed fistula to be in middle lobe.

(Fig. 7B) established the diagnosis of arteriovenous fistula of the right middle lobe. On May 11, 1954, a right middle lobectomy was performed at the Brooklyn Veterans Administration Hospital and the patient made an uneventful recovery. (Table I.)

A low-melting alloy cast of the fistula showed it to occupy most of the medial segment of the right middle lobe. It consisted of a rather stubby, wide pulmonary artery branching into a plexus of intermeshed thin vessels converging into a single dilated vein approximately 1.0 cm. in diameter which also ran, without tributaries, to the right hilus. (Fig. 7C.)

CASE VIII. (Asymptomatic pulmonary arteriovenous fistula, lingular segment, left upper lobe.) On routine chest x-ray (Fig. 8A) a thirty-six year old male schoolteacher (New York Hosp., No. 672688) was found to have increased vascularity along the route of the left descending pulmonary arterial tree. The patient was asymptomatic. On examination he was in good general condition. The heart was normal in size; there were no murmurs; the blood pressure was 120/80; and there was no cyanosis. A bruit was heard after deep inspiration just to the left of the apex of the heart. The arterial oxyhemoglobin was 94 per cent saturated at rest, and the shunt through the pulmonary arteriovenous fistula was calculated to be 15 to 18 per cent of the total pulmonary blood flow.

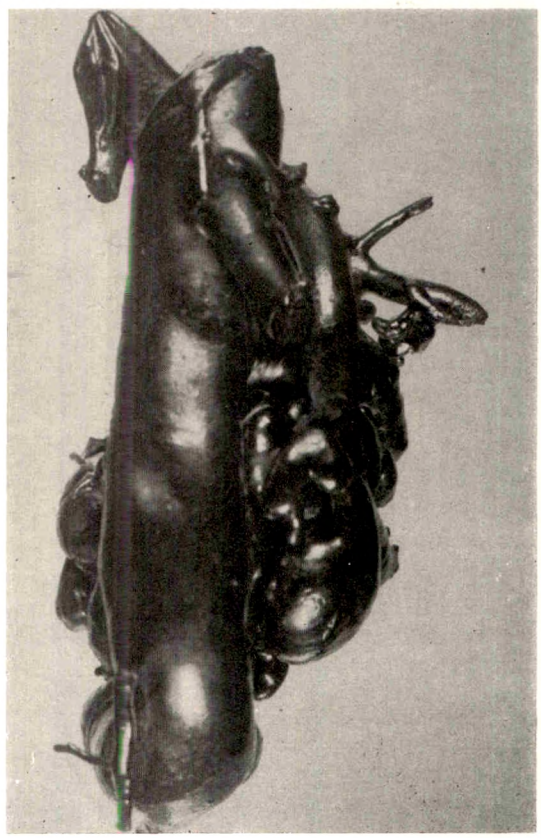
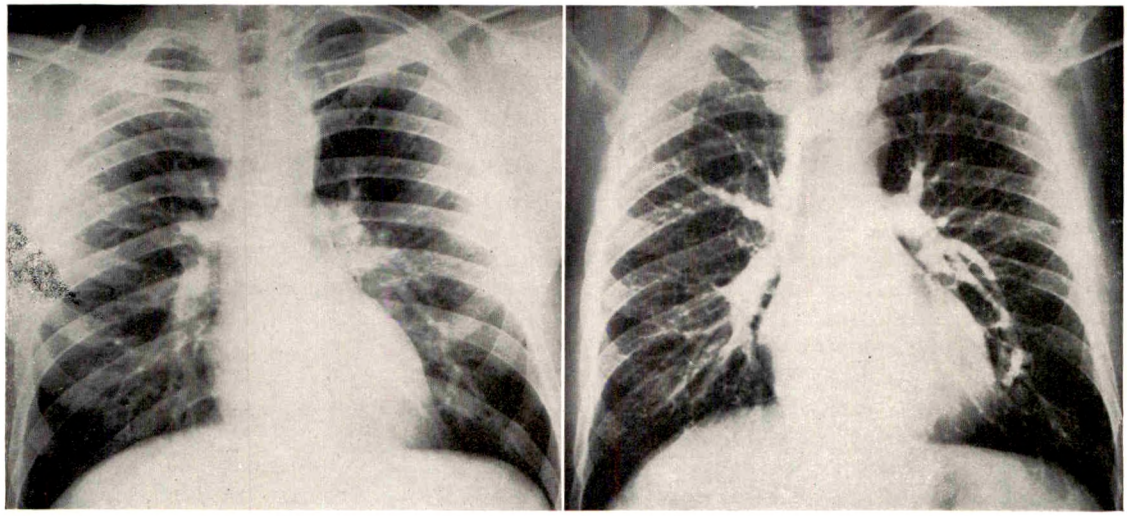
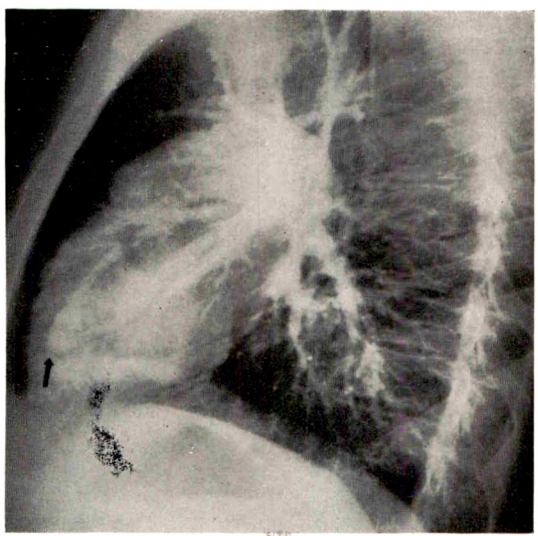


FIG. 7C. Case VII. Right middle lobe arteriovenous fistula in a patient with meningoencephalitis; low melting alloy cast of lesion.



8A 8B



8C

FIG. 8. Case VIII. Lingular segment (left upper lobe) arteriovenous fistula in an asymptomatic male. A, frontal roentgenogram shows increase in vascularity left lower lung field. B, frontal angiocardiogram shows a small arteriovenous fistula. C, lateral angiocardiogram reveals the arteriovenous fistula (arrow) segment with its afferent and efferent branches in the lingular.

Angiocardiography (Fig. 8B and C) revealed a pulmonary arteriovenous fistula in the lingular segment of the left lung. On February 4, 1955, the lingular segment was excised and the patient made an uneventful recovery.

CASE IX. (Pulmonary arteriovenous fistulas, posterior segment right upper lobe and posterior basal segment left lower lobe, associated with Rendu-Osler-Weber's disease.) A fifty year old housewife (New York Hosp., No. 702800) was admitted on February 10, 1955, with complaints of dyspnea and tiredness of ten years' duration. For over twenty years she had been aware of "red spots" on the lips and fingers. As a child she had had frequent nosebleeds; these gradually decreased, recurring only two to three times a year. She had always considered herself well. Six pregnancies were uncomplicated, but during her seventh in 1939 fatigue and dyspnea on exertion began. Anemia was discovered and a "tonic" containing iron was prescribed. She improved and was even able to manage work in a factory. However, two or three times each year she had excessive fatigue, dyspnea and headache which required an iron "tonic" and a month's leave of absence from her job.

In 1949 a routine survey chest x-ray disclosed pulmonary lesions and she was hospitalized for six weeks in a tuberculosis sanatorium. On discharge she was told that she did not have

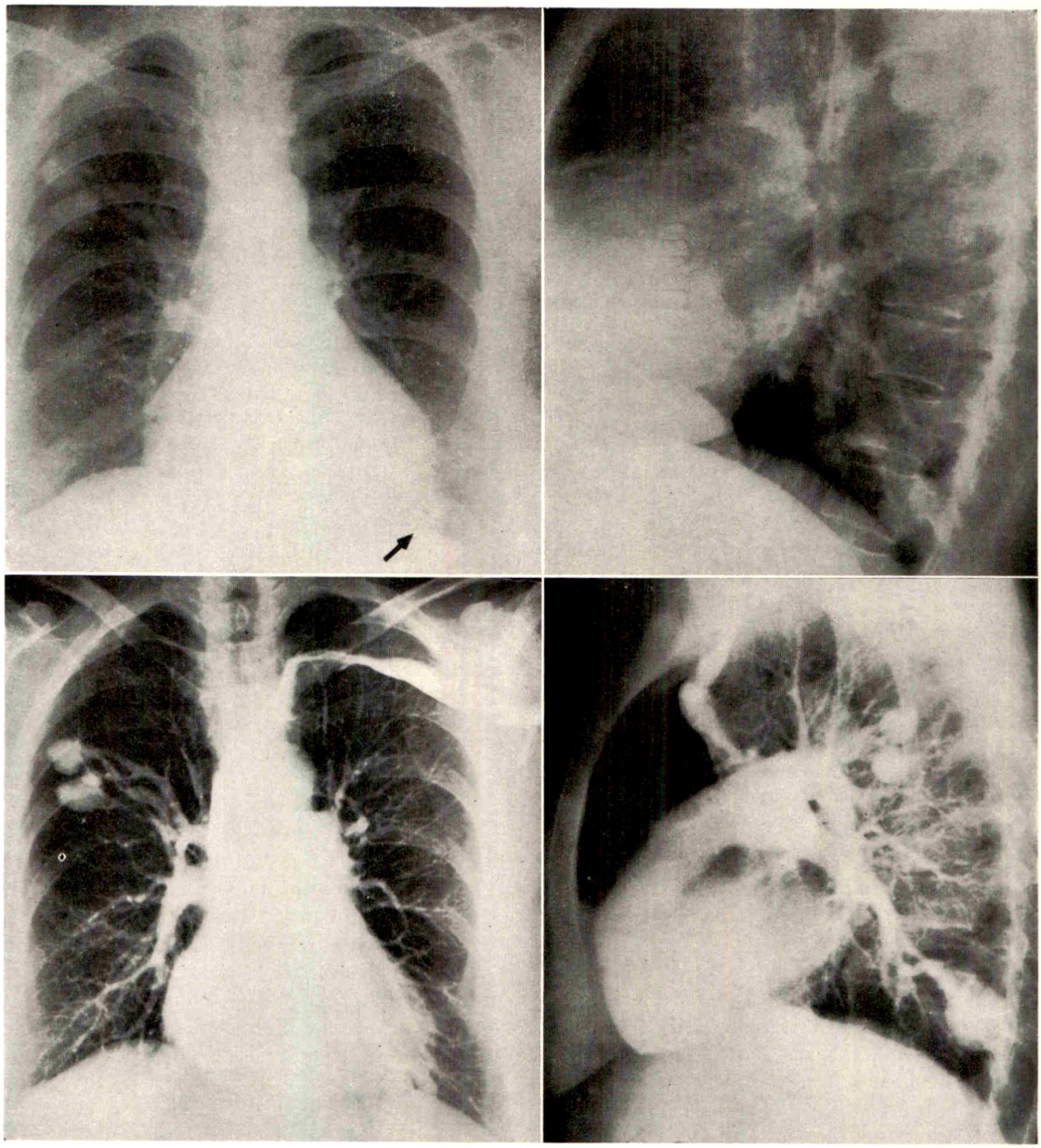


Fig. 9. Case IX. Pulmonary arteriovenous fistulas of the posterior segment right upper lobe and posterior basilar segment of the left lower lobe. A, conventional frontal x-ray shows two rounded opacities in the right upper lobe with hilar connecting afferent and efferent blood vessels. Left lower lobe lesion obscured by heart and diaphragm (arrow). B, lateral, shows the posterior location of the upper lobe densities and another large rounded mass at the base. C, frontal angiocardiogram demonstrates the bilateral pulmonary arteriovenous fistulas. D, lateral angiocardiogram reveals the segmental and posterior location of the fistulas.

tuberculosis; no other diagnosis was made. Fatigue and dyspnea continued and became worse four years before the present admission, requiring a six-month period of rest before she could resume her household duties. A severe upper respiratory infection caused her to be hospitalized recently. A new chest x-ray was made, and this time a physician heard bruits and recognized the presence of bilateral pulmonary arteriovenous fistulas and familial telangiectasis. She was referred to this hospital for treatment. The patient recalled that her

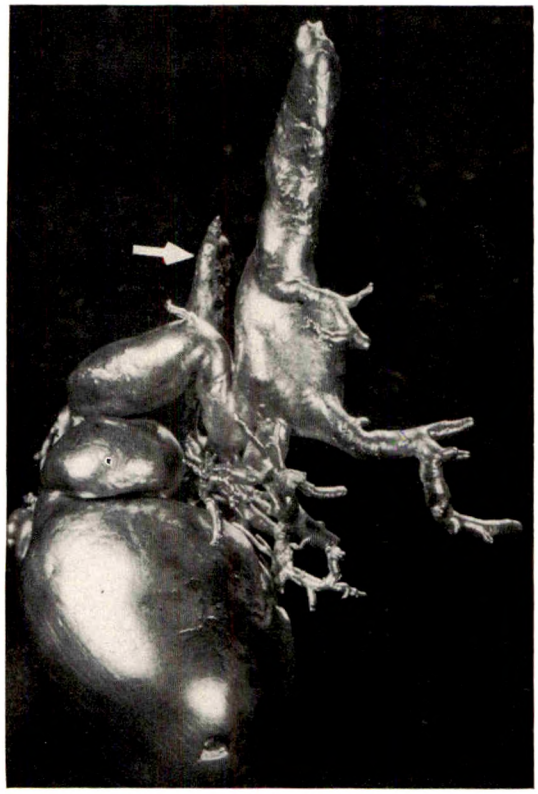


FIG. 9E. Case IX. Pulmonary arteriovenous fistulas of the posterior segment right upper lobe and posterior basilar segment of the left lower lobe; low melting point alloy cast of the left lower lobe fistula.

father and two paternal uncles had "red spots" on the fingers and lips; four siblings and six children are, she believes, free of stigmas of telangiectasis.

On examination the patient was pale, and well developed and nourished. There was slight cyanosis of the lips and finger nails. The skin and mucous membranes showed multiple telangiectasis of lips, buccal mucous membrane, palate, nasal septum, right lower eyelid, finger tips and subungual regions, and toes. There was no clubbing. The heart was normal in size and there were no murmurs; blood pressure was 130/70. The electrocardiogram was normal.

Vascular bruits were heard at the base of the left lung and over the right posterior upper chest. Roentgenograms of the chest (Fig. 9A and B) showed two discrete rounded homogeneous lesions in the posterior segment of the right upper lobe with vascular connections to the hilar structures. The lateral film (Fig. 9B), in addition, revealed a larger rounded dense mass at the base, hidden from view in the frontal projection by the left diaphragm. Angiocardiography in frontal and lateral views (Fig. 9C and D) showed the fistulas. Gastrointestinal and colonic x-ray examinations and proctoscopy failed to reveal gastrointestinal telangiectasis; the stools were free of blood.

Laboratory data (Table 1) showed secondary anemia. The arterial oxygen content in volumes per cent was 14.4, the arterial oxygen capacity 16.5 per cent, and the arterial oxygen saturation 97 per cent. The shunt through the pulmonary arteriovenous fistulas was calculated to be 24 per cent of the total pulmonary blood flow. After three 250 cc. transfusions of packed cells, the hematocrit and hemoglobin were increased. On February 11, 1955, left lobectomy was performed. Postoperative recovery was uneventful.

The excised fistula was injected with low-melting alloy. (Fig. 9E.) A relatively slender branch of the pulmonary artery (arrow) abruptly became dilated and assumed a sinuous course as it entered an ovoid cluster of extremely thin-walled vessels 2.5 cm. in diameter. The fistula was drained by a single tortuous, distended efferent vein approximately 6 mm. in diameter.

DISCUSSION

Pathologic Features

It was long suspected that channels of greater diameter than that of capillaries connect branches of the pulmonary artery and vein. The evidence for this rests on the fact that relatively large particles, such as clumps of tumor cells and Schistosoma cercariae, pass through the lungs into the peripheral circulation. Arteriovenous shunts have been demonstrated in lower animals by Clark and Clark[26] and in the human stomach[27] and skin.[28] By means of ingenious perfusion experiments they were demonstrated in mammalian lungs by Prinzmetal and his group,[29] and their distribution in human lungs was charted by Tobin and Zariquiey.[30] The possible implications of these studies are the subject of a critical report by Brink.[31]

For unknown causes, chiefly congenital, arteriovenous anastomoses and capillaries become engorged and congested, and may do so in any number of patterns. The lesions are usually multiple and may vary in appearance from simple or cirsoid aneurysms of the face, neck or extremities[32] to the more extensive hemorrhagic telangiectasis of Rendu-Osler-Weber's disease.

Capillary or cavernous arteriovenous aneurysms of the viscera, frequently of the lungs, are but two kindred forms of a versatile disease. The multiplicity of the anomalies is important: In their survey of the eighty-five recorded cases of congenital pulmonary arteriovenous fistulas Sloan and Cooley[1] reported two or more lobes to be involved in twenty-seven patients, and both lungs in twenty-one. Figure 9 illustrates a case in which there were arteriovenous fistulas in the right and left lungs.

The gross appearance of the six resected fistulas in our series was in no way unusual. All were thin, globular sacs lying peripherally just beneath the pleura, supported by a rather frail stroma. In none of our specimens were calcified plaques found.

The blood supply and drainage of congenital arteriovenous fistulas is capricious and of the greatest importance at the time of thoracotomy. The varied possibilities are indicated in Figures 5D, 6D, 7C and 10. Clearly, the fistula is not simply a distended sac fed by an end artery and drained by a vein, but rather one part of an intricate anomaly affecting not only the principally involved lung but also neighboring vessels, chest wall and often the opposite lung as well. Communications may exist between terminal arteriole and vein;[33] but the artery and vein often follow an unconventional course. The artery may emerge from a normal adjacent lobe,[34] from the aorta[35] or from intercostal arteries.[6] Veins from normal adjacent lung may flow into the aneurysmal sac,[36] and wholly bizarre vessels may drain the fistula. It has been suggested, as in our Case VI, that bronchial arteries may contribute to the fistula[37] and a parallel series of direct shunts may connect the pulmonary artery and vein.[1] (Fig. 10.)

A limited amount of information about vascular lesions may be obtained by injecting them with radiopaque solutions such as barium, bismuth oxychloride,[38] cinnabar[39] or latex[40] and then making radiographs of the specimen before sectioning it. In this way the tissue may be examined histologically if the pathologist so desires. Yet the gross anatomy of these lesions and its continuity offers more information than microscopic examination, and the injection-corrosion method best demonstrates the origin, size and relationship of the vessels making up the fistula. Four of our six specimens were reconstructed by injecting them with a metal alloy of low melting point[41] and digesting the surrounding tissue with a strong alkali. It would perhaps have been as easy to inject vinylite plastic, as suggested by Liebow,[42] a method offering the additional advantage of color identification to a three-dimensional cast.

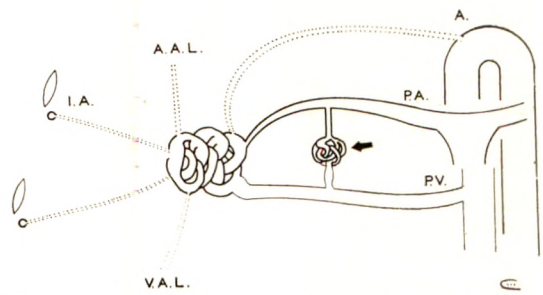


FIG. 10. Schematic drawing of anomalous connections of a pulmonary arteriovenous fistula. Continuous lines represent the pulmonary artery (P.A.) entering and the dilated branch of the pulmonary vein (P.V.) leaving the fistula. Dotted lines indicate anomalous branches from the aorta (A), the artery from the adjacent lobe (A.A.L.), vein from adjacent lobe (V.A.L.) and intercostal artery (I.A.). Small collateral shunts (arrow) may also be present proximal to the fistula, connecting pulmonary artery and vein as indicated above.

Clinical Features

Incidence, Sex and Race. Pulmonary arteriovenous fistulas are uncommon despite the increasing reports in the literature. In this Center only nine cases were collected in a total of 2,000 patients studied angiocardiographically during an eight-year period. The nine cases represent a select group: Two (Cases VII and VIII) were referred for more complete angiocardiographic study after the diagnosis had been established by angiocardiography elsewhere; two others (Cases III and IV) were referred from the Tuberculosis Clinics of the New York City Department of Health. Only five cases were found in The New York Hospital (Cases I, II, V, VI and IX) during the years 1950–1955. As in Sloan and Cooley's report,[1] there was no significant sex preference; six patients were female and three were male. The ages varied between nineteen and sixty-seven years and all patients were of the white race.

Familial Tendency and Relation of Pulmonary Arteriovenous Fistulas and Hereditary Hemorrhagic Telangiectasis. Two siblings (Cases IV and V) were among the eight cases. (Table I.) This is the third set of siblings reported in the literature.[16,18] Pulmonary arteriovenous fistulas in father and son,[43] in daughter and probably father,[1]

and mother and son[10] have also been reported recently. A history of hemangiomas and telangiectasis (Rendu-Osler-Weber's disease) in other members of the family in our eight patients could only be elicited once (Case IX). In the literature, however, 15 per cent had a familial history of this disease.[1] Two of the patients (Cases I and IX) had associated hemangiomas, and over 40 per cent of the reported cases of pulmonary arteriovenous fistula also had telangiectatic lesions elsewhere in the body.[1] These findings clearly establish the familial nature of pulmonary arteriovenous fistula and the close relation to hereditary hemorrhagic telangiectasis.

Symptoms and Physical Findings. Only one patient (Case III, Table I) had dyspnea, cyanosis and clubbing of the fingers and toes. The findings were due to anoxemia and resulted from involvement of the whole left lower lobe (the largest in our series) in the arteriovenous fistula. (Fig. 3.) This is in contrast to the forty-four instances of dyspnea among the seventy-nine cases collected from the literature by Sloan and Cooley,[1] and suggests that only the large and more advanced pulmonary arteriovenous fistulas have hitherto been recognized. The absence of cyanosis and polycythemia in Case VI may be due to the bronchial arterial communication with the fistula, disclosed by the operation. Either the systemic arterial connection may be sufficient to prevent unsaturation of blood or else the increased bronchial arterial pressure may alter and even reverse blood flow in an arteriovenous shunt. The absence of polycythemia in Case IX is explained by the chronic anemia.

Two patients (Cases I and VII) were admitted because of acute cerebral symptoms, one with a brain abscess and the other with acute meningoencephalitis. Neurologic disturbances are common in patients with pulmonary arteriovenous fistulas and have been usually attributed to cerebral anoxemia or to vascular thrombosis associated with the polycythemia.[1] However, as in Case I, brain abscesses are being found more frequently.[4,5,9,23,44]

Three patients (Cases IV, V and VIII) were asymptomatic. Two others (Cases II and VI, Table I) had diseases unrelated to the pulmonary arteriovenous fistulas which were incidental findings. Only the patient with dyspnea, cyanosis and clubbing of the digits and polycythemia (Case III) was poorly nourished. The heart and electrocardiogram were normal in all instances except Case II in which rheumatic heart disease was also present. The finding of a heart of normal size in patients with pulmonary arteriovenous fistulas is explained by the absence of increased pulmonary vascular resistance.[1,45,46]

Vascular bruits over the site of the pulmonary arteriovenous fistulas were heard in all but one patient (Case II). The murmurs varied from a faint systolic sound accentuated by deep inspiration to a loud harsh systolic blow; in one instance the bruit was present during diastole also. Accurate localization of the fistula by roentgenography in frontal and lateral views aided in eliciting the auscultatory signs.

Roentgen Features

The most constant finding in pulmonary arteriovenous fistulas is the presence of an abnormal shadow in the conventional chest roentgenogram.[24,34] The fistulas may vary in size and shape from minute circular densities (Figs. 2A and 5A) to large sharp oval lesions. (Figs. 1A, 6A and 9A.) Vague and ill defined edges may simulate pulmonary infiltrates. (Figs. 3A and 4A.) At times, localized increase of the hilar pulmonary vasculature may suggest an arteriovenous fistula (Figs. 7A and 8A), especially when lateral or oblique views indicate that the increased vasculature is related to the segmental or lobar pulmonary blood supplies. Another feature, the afferent pulmonary arterial and efferent pulmonary venous channels connecting with a pulmonary lesion, may be evident. (Figs. 1A and B, 2A, 6A and 9A.) Rarely, anomalous pulmonary venous drainage from the right lung into the inferior vena cava may simulate a pulmonary arteriovenous fistula.[47,48] Calcifications in pulmonary arteriovenous fistula are uncommon and have been reported in only three instances.[1]

Pulmonary arteriovenous fistulas may occur in any segment, subsegment or lobe. In our series the left lower and right middle lobes were involved respectively in two instances, while in the seven other cases the lesions were segmental. (Table I.)

Fluoroscopy permits recognition of vascular pulsation of pulmonary arteriovenous fistulas and their vascular connections, but in none of our cases was this phenomenon observed. More important are the changes in size of the lesions during respiratory maneuvers.[34] With the

Valsalva procedure (expiration against the closed glottis) the intrathoracic pressure is raised, blood flow diminishes in the thorax and the fistula becomes smaller. The Müller procedure (deep inspiration against the closed glottis) decreases the intrathoracic pressure and more blood fills the fistula and makes it larger.

Body section roentgenography has been highly recommended for the diagnosis of pulmonary arteriovenous fistulas.[1,24,34,44] In some instances it may be expected to supplement conventional roentgenography and disclose vessels connecting with the fistula. However, disease processes like bronchiectasis and chronic pneumonia may be exaggerated and reveal patterns that simulate pulmonary arteriovenous fistulas.[49]

In the roentgenographic differential diagnosis, primary or secondary lung tumors, bronchiectasis and tuberculosis are some of the conditions to be considered. One patient (Case II) was suspected of having a metastatic lung lesion because of the presence of a brain tumor. Bronchiectasis was diagnosed in Case III because of clubbing of the digits and haziness of the left lower lobe. Another patient (Case IV) was thought to have a left infraclavicular tuberculous infiltrate, while in Case VI a primary lung neoplasm or hamartoma was suspected.

Angiocardiography. There is general agreement that angiocardiography provides the definitive diagnosis of pulmonary arteriovenous fistulas.[1,2,24] Robb[50,51] first and Linskog and co-workers[36] later called attention to the importance of making large (14 by 17 inch) roentgenograms in order to prevent overlooking multiple and bilateral pulmonary arteriovenous lesions. Apparently, minute pulmonary telangiectasis may not be readily discernible by angiocardiography.[52] However, small arteriovenous fistulas in pulmonary segments are easily recognized. (Figs. 4, 5, 7 and 8.) The afferent and efferent vascular connections of pulmonary arteriovenous fistulas may be difficult to visualize with conventional roentgenography but are readily seen with angiocardiography. Multiple serial films are sometimes desirable for complete study of the lesion during the arterial and venous filling stages but usually two large films taken with a standard stereo-cassette changer[53,54] at 2½ and 6 seconds after the beginning of the injection will show the pulmonary arterial and venous components of the fistula. Involvement of the chest wall by pulmonary arteriovenous fistulas has been reported.[6] With angiocardiography this complication might have been recognized.

In the literature there are two reports of untoward reactions following angiography in patients with pulmonary arteriovenous fistulas. A fatality[55] occurred after the injection of 35 cc. of the contrast agent through a cardiac catheter with tip in the right atrium. Another patient[56] recovered after severe hemoptysis which followed injection of contrast material through a cardiac catheter placed in the main pulmonary artery. The recent report of rupture of the heart by injection of the contrast substance through a cardiac catheter during selective angiography[57] emphasizes the danger of injecting contrast substances directly into the cardiovascular system via catheters.

In marked contrast is our experience with the intravenous method of angiocardiography. In all, 2,573 injections of contrast material have been made in 1,939 patients during the period from October, 1946 to 1954. Only one death occurred, this in a fifty-six year old woman who had a pericardial effusion probably secondary to irradiation fibrosis. This record, in a series containing many patients seriously ill with heart and lung disease, fully justifies the use of angiocardiography, especially in pulmonary arteriovenous fistulas, for in this disease preoperative information in regard to bilateral or multiple involvement of the lungs is essential for the planning of the operation and conservation of lung tissue. In spite of recent warnings,[24] cardiac failure and/or hypertension are not contraindications to angiocardiography.

Treatment

There is general agreement that the best treatment for pulmonary arteriovenous fistulas is surgical excision of the lesions, with removal of as little lung tissue as possible in order to preserve pulmonary function, especially if multiple lesions are present. There are many reports in the literature of the disappearance of dyspnea, cyanosis and polycythemia following surgery.[1,12,15,16] Surgery is even advocated as a prophylactic measure in asymptomatic patients.[25] The risk of lobectomy or segmental resection is far less than the morbidity and mortality of complications like brain abscess,[4,5,9,23,44] hemoptysis[1,2,58] and cerebral thrombosis due to polycythemia.[1]

Too little time has elapsed to know if there will be recurrence of pulmonary arteriovenous

fistulas in other portions of the lung after surgery. Two cases are on record[1] in which pre-existing pulmonary arteriovenous fistulas enlarged after surgical excision of associated pulmonary fistulas. The recent finding of multiple minute pulmonary telangiectasis on lung biopsy[52] indicates the futility of lung surgery in such a condition.

SUMMARY

Pulmonary arteriovenous fistulas are hereditary hemangiomatous malformations of the pulmonary vascular bed. Almost 40 per cent of the reported cases also have hemangiomas elsewhere in the body, indicating that a close relationship to familial hemorrhagic telangiectasis (Rendu-Osler-Weber's disease) exists.

When pulmonary arteriovenous fistulas are large and do not have systemic arterial connections, dyspnea, cyanosis, clubbing of the digits and polycythemia result from unsaturation of the blood. Hemoptysis may occur when there is rupture of the paper-thin pulmonary vascular anomaly. There is also a high incidence of cerebral symptoms due either to cerebral thrombosis, secondary to the polycythemia, or to infection, especially brain abscess. The heart remains normal in size because pulmonary arteriovenous fistulas do not cause increased pulmonary vascular resistance.

In our series of nine cases the classical syndrome of cyanosis, clubbing of digits, polycythemia and a vascular murmur heard over the pulmonary fistula was present in one case only. One patient with dyspnea, fatigue and familial telangiectasis had bilateral pulmonary arteriovenous fistulas; chronic anemia prevented intense cyanosis and polycythemia. In three instances the patients were asymptomatic; in two the pulmonary arteriovenous fistula was an incidental finding; in the remaining two, acute cerebral conditions (brain abscess and meningoencephalitis) were the presenting symptoms. Vascular bruits were heard in eight patients. All patients had abnormal chest roentgenograms.

The diagnosis of pulmonary arteriovenous fistula can often be made by conventional roentgenography, particularly if the index of suspicion is high. Pulmonary densities with hilar vascular connections localized in lobes or segments of the lung, or increased hilar vascularities, should arouse suspicion of an arteriovenous fistula. Change in size of the lesions after respiratory maneuvers (Valsalva, Müller) and delineation of the afferent arterial and efferent venous connections by body section radiography may be confirmatory. Angiocardiography, however, establishes the diagnosis and is recommended for preoperative evaluation.

The treatment of pulmonary arteriovenous fistulas is surgical excision, with preservation of as much lung tissue as possible in order to preserve pulmonary function, especially if multiple lesions are present. Surgery is usually curative. It is recommended by some even in the asymptomatic case since the risk of brain abscess, fatal hemoptysis or development of cerebral thrombosis when polycythemia occurs is far greater than the morbidity and mortality of lobectomy or segmental resection.

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Pulmonary Lesions in "Rheumatoid Disease" with Remarks on Diffuse Interstitial Pulmonary Fibrosis*

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"RHEUMATOID disease" is a more descriptive term than rheumatoid arthritis since it expresses a widespread affliction involving not only the locomotor system but also other parts of the body.[1] For reasons to be given later, there is much to recommend this all-inclusive designation and it will be used in this sense in the following pages.

In 1948 Gruenwald[2] described a case of "rheumatoid disease" with visceral lesions and commented that this combination had not been reported previously except for passing mention of nodules in subcutaneous tissues, skeletal muscles and nerve trunks, also scars of rheumatic heart disease. The case cited was found to have granulomatous lesions in the heart, pleura and capsule of the spleen, indicating that all serous membranes may be involved. The Ninth Rheumatism Review,[3] published in 1948 and containing a comprehensive summary of the English and American literature on the subject, refers to studies by Baggenstoss, Rosenberg and Hench[4,5] in which are described thirty cases of "rheumatoid disease" examined at autopsy. A high incidence of cardiac lesions, indistinguishable from those of rheumatic fever was found, also a relative frequency of microscopic renal lesions. Although pneumonia, chronic suppuration, embolism and massive collapse of the lung were the most common causes of death, the authors could find no distinguishing pulmonary lesions which could be correlated with the "rheumatoid disease." Nor could they find any relationship with the frequent finding of fibrous pleural adhesions which were present in twenty-two of the thirty cases. The Tenth Rheumatism Review, published in 1953,[6] contains three references[7–9] in which are described five cases of "rheumatoid disease" with pulmonary findings. In 1954 Harris[10] was able to collect references to a total of twenty cases of pulmonary lesions associated with "rheumatoid disease" and cited a case of his own. Five of these cases were studied at autopsy, two by Ellman and Ball and three by Christie.[11] Two additional cases, with autopsy examinations showing pulmonary lesions, have been reported, one by Bevans and co-workers[12] and the other by Katz and Auerbach.[13] The literature on the subject appears to be growing rapidly.

With increasing use of routine chest roentgenography, changes are being discovered in the lungs of patients with "rheumatoid disease" indicating that these organs are involved quite frequently and early in the course of the disease. Inasmuch as the lungs, as visualized in chest x-rays, often serve as mirrors of what is going on in other parts of the body,[14] roentgenography offers a useful means of investigation of obscure systemic diseases associated with pulmonary manifestations. Points of similarity or dissimilarity between diseases, featured by lung changes which are demonstrable roentgenologically, are often easily recognized and the significance of such changes, at times, better appreciated in serial chest x-rays in the light of the clinical findings than at autopsy. As to the applicability of chest roentgenography to the study of pulmonary manifestations of "rheumatoid disease" and to related collagen diseases, it may be pointed out that the lungs possess a dual blood supply, an immense interstitial stroma and a broad pleural serosa, tissues which are most expressive of mesenchymal disorders.

Within a space of two months the writer had occasion to observe three patients with acute

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"rheumatoid disease" with characteristic articular manifestations in whom the chest x-ray findings were in keeping with a systemic disturbance. In two cases it was possible to obtain lung biopsies for histologic study. Since the roentgenologic and pathologic findings in the cases studied resembled in some respects those seen in several other members of the collagen group of diseases, also in occasional instances of diffuse interstitial fibrosis of unknown etiology, reference will be made to pertinent findings in these groups of cases. The last mentioned is currently being studied by Lubliner and the writer.[15]

CASE REPORTS

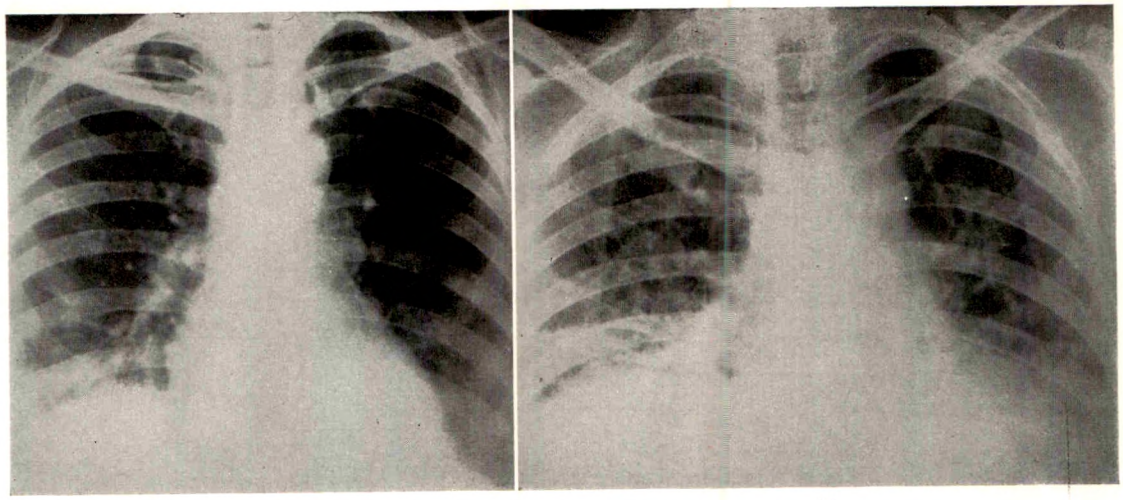
CASE 1. A. R., a forty-nine year old white female, was subject to frequent upper respiratory infections as a child. Repeated chest x-rays failed to reveal any abnormalities. At the age of twenty-eight the patient had an ovary, fallopian tube and appendix removed. She continued to have her menses regularly. In July, 1949, the patient developed symptoms which were ascribed to pneumonia. A chest x-ray revealed faint haziness in both lower lobes but no distinct parenchymal involvement. The condition cleared with penicillin treatment. During the following two years the patient experienced menopausal symptoms for which she received hormonal treatment.

In June, 1952, after an emotional disturbance, the patient developed generalized urticaria of the hands and face, with swelling and pruritus, also pain, redness and swelling of the joints beginning in the toes and spreading to the ankles, upper extremities and other joints. The patient also experienced deep muscle pain. The menses ceased abruptly. On August 23, 1952, the patient was hospitalized for treatment of her "rheumatoid disease." Chest x-rays showed no significant changes from those noted three years previously. (Fig. 1A.) The patient was treated with salicylates and discharged after a week, somewhat improved.

Two months later there was recurrence of rheumatoid symptoms and at this time the patient complained of shortness of breath which seemed worse during the periods of aggravation of the joint pains. In January, 1953, because of increasing severity of the arthritic symptoms, the patient was again hospitalized. In addition to the dyspnea and joint pains, there were now present anemia, four-pillow orthopnea, an enlarged liver and ankle edema. A chest x-ray disclosed, for the first time, soft irregular mottlings in both lungs; also diffuse, interstitial striations, especially in the lower lobes, possibly a small amount of fluid at both bases and prominence of the horizontal fissure on the right. The right leaf of the diaphragm was displaced upward. (Fig. 1B.) Repeated chest x-rays taken during the following months showed no significant changes except for greater accentuation of the interstitial striations and less soft mottling. The patient was treated with digitoxin, iron and cortisone. The swelling, redness and pain disappeared from the hands. For about nine months the patient continued to take cortisone periodically, also mercurials and salicylates to relieve the joint pains.

In December, 1953, the joint symptoms recurred and the patient was rehospitalized. On this occasion she was given penicillin but developed a generalized urticaria and was given cortisone for relief. A chest x-ray showed accentuation of the diffuse pulmonary infiltrations. Several weeks later the patient was admitted to the New York Hospital. Cardiopulmonary functional tests were performed in this institution and the results, furnished through the courtesy of Dr. I. Steinberg, revealed the following: Cardiac catheterization showed moderate pulmonary arterial hypertension compatible with diffuse pulmonary fibrosis. An angiocardiogram revealed compression of the pulmonary vessels and slight enlargement of the pulmonary artery suggestive of elevated pulmonary artery pressure. The left ventricle was slightly enlarged. Pulmonary function tests revealed impairment of ventilatory capacity indicative of pulmonary fibrosis of a diffuse variety. Other laboratory findings were similar to those obtained several months later at the Montefiore Hospital where the patient was admitted on February 27, 1954, and remained until discharge March 20, 1954.

The patient appeared chronically ill, dyspneic even at rest, and complained of generalized joint pains requiring salicylates and sedation. The temperature was 99.6°F., pulse 120 and regular, respiratory rate 20 and blood pressure 130/90. The skin of the face revealed a macular erythema more marked over both cheeks. The thyroid was not enlarged; there was no venous distention. The extremities showed moderate fusiform enlargement of the interphalangeal joints of the hands and wrists which were puffed and painful on motion. A slight deformity of the right hand was ascribed by the patient to long-



1A 1B

FIG. 1. (Case 1.) A, August 25, 1952; faint haziness over both lower lung fields; possibly pleural reaction at bases. B, chest x-ray, February 16, 1953; soft irregular mottlings throughout both lung fields and faint reticular infiltrations; prominent horizontal fissure on right; possibly small collections of fluid at bases; right leaf of diaphragm displaced upward.

standing ulnar nerve injury. The knees and ankles were also swollen and painful. There was impaired percussion over both lower lobes, especially over the right base. Auscultation revealed rales over the entire chest, chiefly posteriorly, and at the bases where a coarse friction rub could be heard. The friction rub was present throughout the patient's stay at the hospital and in follow-up examinations. The heart rate was regular with a soft systolic murmur at the left border. P_2 was accentuated. The liver was palpable three finger breadths below the right costal margin and was not tender. The spleen was not felt.

Chest x-rays showed accentuation of diffuse interstitial striations throughout both lung fields but, on the whole, the changes were similar to those seen in previous films. (Fig. 1C.) X-rays of the hands, shoulders, feet and other joints revealed moderate osteoporosis, especially of the hands, narrowing of joint spaces but no erosion. Laboratory data included: urine negative; hemoglobin 11 gm. per cent; red blood cells 4,500,000 per cu. mm.; white blood cells 8,050 per cu. mm. with a normal differential. Erythrocyte sedimentation rate was elevated; no lupus erythematosus cells in the peripheral blood. Serum albumin 3.6 gm. per cent, serum globulin 4.2 gm. per cent. An electrocardiogram showed old posterior wall infarction with right bundle branch block. Basal metabolism +47 per cent; repeated +34 per cent; cephalin flocculation

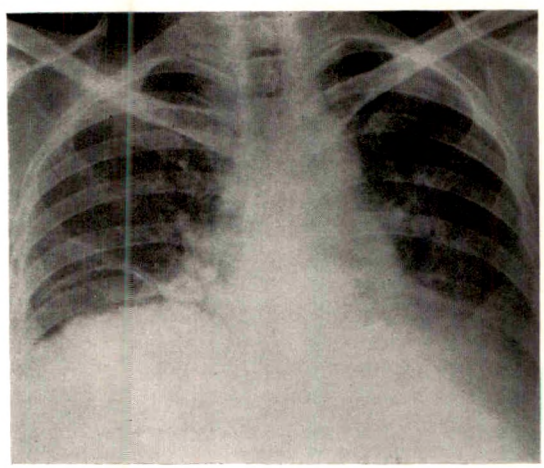
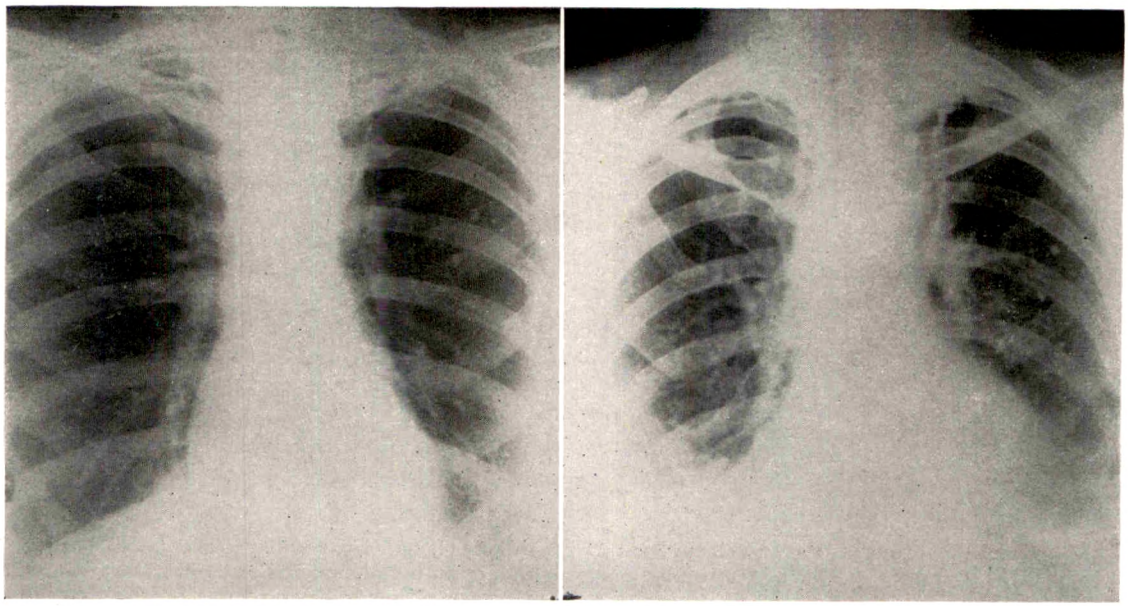


FIG. 1C. Chest x-ray (Case 1), February 23, 1954; diffuse interstitial striations throughout both lung fields; irregular density, left lower chest.

1+, thymol turbidity 18. Other blood examinations showed no significant findings. Repeated examination of the sputum failed to reveal acid-fast organisms. Skin and muscle biopsies which had been obtained previously at the New York Hospital had shown no abnormalities.

The patient continued to have dyspnea, worse when the joint pains were more severe. Salicylates and sedatives gave partial relief. Prior to the institution of hormonal treatment spirometry was done. The vital capacity was 1,000 cc. with a maximum breathing capacity of 43 L. per minute. After ten days' treatment with corti-



2A 2B

FIG. 2. (Case II) A, chest x-ray, October 18, 1949; scattered calcific foci in left lung and thickened pleura on that side; the right lung field is clear. B, chest x-ray, May 21, 1954. In addition to calcified foci in left lung seen in previous chest x-rays, there are now diffuse interstitial striations in both lungs, especially the right lung; there was pleural reaction at both bases.

cotropin the vital capacity rose to 1,800 cc. and the maximum breathing capacity to 73 L. per minute. The patient improved symptomatically and was able to knit which she could not do previously. The linear infiltrations in the lungs appeared slightly less pronounced but, on the whole, the x-ray findings were unaltered. After discharge from the Montefiore Hospital the patient continued to receive decreasing doses of corticotropin supplemented with, and later replaced by, cortisone. After four months the hormonal treatment seemed to be decreasingly effective and the patient required salicylates to relieve the joint pains, also inhalations of vaponefrin to relieve the paroxysms of dyspnea. At the time of this writing (August, 1954) the disease appears to be slowly progressive. The patient has lately noted a rash over the upper chest and arms associated with itch at the time of recurrence of joint pains and dyspnea.

CASE II. H. D., a fifty-eight year old white female, was found to have pulmonary tuberculosis of the left upper lobe at the age of thirty-two. The disease underwent spontaneous arrest. Thirteen years later the disease relapsed and the sputum contained acid-fast bacilli. A pneumothorax was induced on the left side and after one year of collapse therapy the lung was released. Following this the patient felt well and attended to her household duties. Repeated chest x-rays showed a calcified scar in the left apex, scattered calcific foci in the left upper lobe and a thickened pleura on that side. (Fig. 2A.) The sputum was repeatedly negative for acid-fast bacilli.

In November, 1952, the patient developed pain in the right lower chest associated with slight cough but no fever. A chest x-ray disclosed soft irregular infiltrations at the right base suggestive of pleurisy or atypical pneumonia rather than reactivation of tuberculosis. The remainder of both lung fields was unchanged. Sputum examinations continued to be negative for acid-fast bacilli. The patient was treated with aureomycin and penicillin. On one occasion she received sulfonamide but developed a skin rash and itch on the tenth day and the medication was discontinued. The rash cleared spontaneously in about two months but recurred in mild form on later occasions. In December, 1952, the patient had a recurrence of right-sided chest pain, less severe than the first episode, and she was treated with bed rest alone.

In April, 1953, as the chest pain was receding, the patient developed painful swellings of joints, beginning at the wrists and spreading to the

elbows, shoulders and spine. The skin felt hot and there was pain on motion. The patient was treated with salicylates during the following year. On April 26, 1954, the patient consulted the writer because of recurrence of chest pain and rheumatoid symptoms. At this time she also had cough, night sweats, fever and general malaise. She had noticed dyspnea shortly after the onset of the original pleurisy which had become progressively worse. The patient stated that she had always been in good health except for a transient period of hypertension during her menopause. Except for the urticarial reaction after the administration of sulfonamide, the patient had had no other stigmata of hypersensitivity. A chest x-ray now revealed diffuse reticulations throughout both lung fields with evidence of a pleural reaction at both bases, in addition to the evidence of inactive tuberculosis noted previously. (Figs. 2B and 2C.) In view of the past history of tuberculosis, the patient was given 300 mg. isoniazid daily to note whether the drug would have any effect on the joint symptoms. At the end of two weeks the patient complained that the rheumatic pains had become worse and the medication was discontinued.

The patient was admitted to the Montefiore Hospital on May 27, 1954. Physical examination revealed a sallow, chronically ill woman somewhat dyspneic even at rest. The temperature was 100°F., pulse 100, respiration rate 20 and blood pressure 140/80. The interphalangeal joints of the fingers, also the wrists, elbows and shoulders, were swollen and painful on motion. The joints of the lower extremities were not affected. Examination of the chest disclosed the heart to be slightly enlarged, rhythm irregular with no murmurs. Auscultation of the lungs revealed many rales at both bases. A pleural friction rub was heard posteriorly, chiefly over the right lower chest.

Chest x-rays revealed the findings noted previously. X-rays of the hands and elbows revealed moderate osteoporosis of the bones of the carpus and swelling of the surrounding soft tissues. There were no productive arthritic changes. An electrocardiogram showed right axis deviation and multiple premature beats of the ventricle. Other laboratory findings were Mantoux 2+in 1:100 O. T. dilution, hemoglobin was 10 gm. per cent, red blood cells 3,710,000 per cu. mm., white blood cells 7,750 per cu. mm. with a normal differential The erythrocyte sedimentation rate was accelerated. Serum albumin was

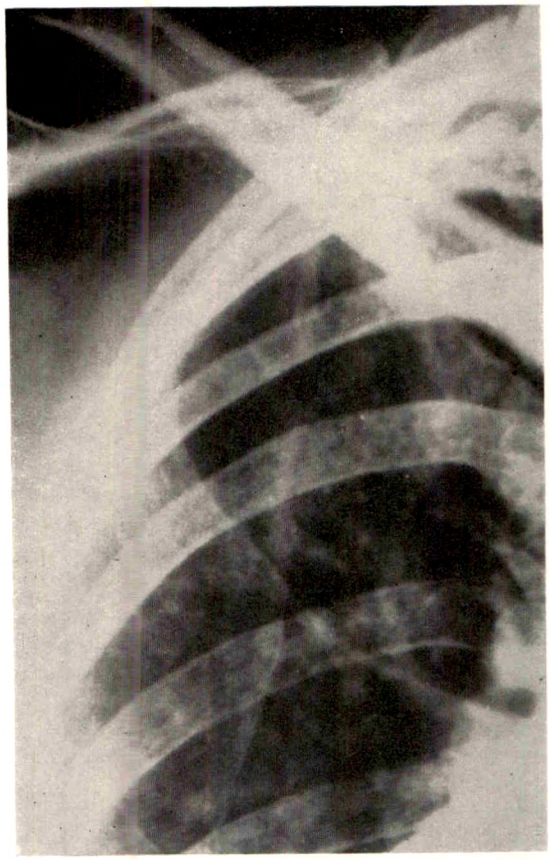


FIG. 2C. Chest x-ray (Case II), May 28, 1954; enlargement showing detail of right upper lobe.

4.2 gm. per cent, serum globulin 3.3 gm. per cent, fasting blood sugar 90 mg. per cent, blood urea nitrogen 11.2 mg. per cent, uric acid 4.6 mg. per cent, serum calcium 10 mg. per cent, serum phosphorus 3.4 mg. per cent, serum cholesterol 232 mg. per cent, L.E. preparations of the blood were negative, liver function tests, cold agglutinins and routine serologic tests were also negative. Repeated examinations of the sputum failed to reveal the presence of acid-fast bacilli.

Because of the persistence of joint pains and low-grade fever which did not respond to salicylates, it was decided to give the patient a trial of cortisone. Prior to the administration of the latter, a biopsy of the right lower lobe was done by Dr. Morris Rubin on June 10, 1954. The specimen was examined by Dr. J. Hasson. The findings were as follows: the pleural surface of the specimen was focally covered with fine adhesions. The pleura was noted to move freely on the underlying lung parenchyma suggesting

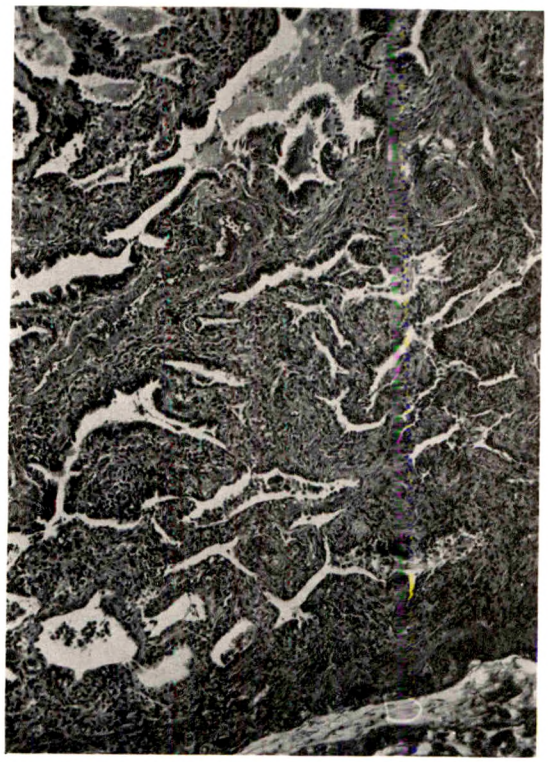


FIG. 2D. Lung biopsy (Case II) showing marked thickening of interalveolar septa; distortion, narrowing and elongation of remaining alveolar spaces; hyperplasia of alveolar lining cells; thickened wall of blood vessel seen in upper right field.

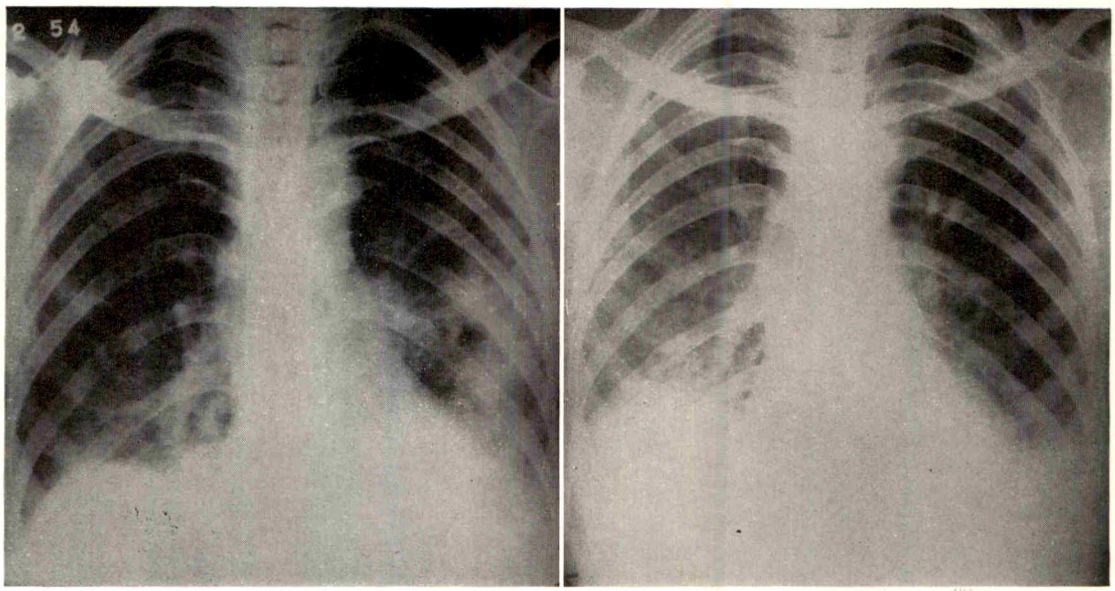
edema of the subpleural connective tissue. On section the parenchyma appeared collapsed and gray-pink with focal specks of anthracotic pigment. One area of hemorrhage was encountered which was fairly discrete and round and measured 0.6 cm. in diameter. Another focal area appeared as solidified parenchyma suggestive of consolidation. This area appeared gray-white and poorly defined.

Microscopic examination disclosed a markedly altered pulmonary parenchyma with marked fibrosis of the alveolar septa and distortion of the alveolar spaces. (Fig. 2D.) These spaces were focally lined by a cuboidal epithelium and filled with a mucinous exudate containing mononuclear cells. The interstices were thickened by an increased amount of fibrous tissue and a scattered exudate of mononuclear cells consisting mainly of lymphocytes and scattered plasma cells and histiocytes. The density of this exudate varied from zone to zone. In some areas small dense aggregates of lymphocytes were seen in the interstices. Occasional bronchioles were seen which contained plugs of mucinous material containing mononuclear cells including exfoliated epithelial cells. No intact lung parenchyma was seen in any of the sections. There was no evidence of tuberculosis. The blood vessels showed a patchy, hyperplastic sclerosis of the small arteries and special stains disclosed that this was due mainly to subintimal fibrosis with a lesser element of hyperplasia of the media. Thin, vascular, fibrous adhesions were seen attached to the pleura which was somewhat thickened. The diagnosis was diffuse interstitial fibrosis of the lung with hyperplastic sclerosis of small arteries.

The patient was discharged from Montefiore Hospital on June 16, 1954, slightly improved. Late in June, 1954, the right knee and both ankles became swollen and painful. The patient was given cortisone 100 mg. daily and after one month there was considerable symptomatic improvement. Auscultation still revealed a pleural friction rub, less marked than on previous examinations; other physical findings were unchanged. Chest x-rays in August, 1954, have shown slight regression in the diffuse interstitial markings.

CASE III. E. T., a forty-six year old Negro female, became ill on December 20, 1953, with fever, cough and dyspnea, also painful, swollen joints. The patient was bedridden at home for two weeks and was treated with penicillin. Although not fully recovered, she returned to work as a domestic. On February 20, 1954, there was a return of fever, cough and painful joints; at this time, the patient also had chest pain and, on one occasion, spat up a small amount of blood. The patient was hospitalized on March 11, 1954. Examination revealed an acutely ill, dyspneic woman; temperature 103°F., pulse 110, respiration rate 24 and blood pressure 100/80. Both hands, especially the left, showed fusiform, tender swellings of the interphalangeal joints. The right knee was also swollen and tender. Examination of the chest revealed dullness over both sides and rales at the bases. The heart revealed no abnormal findings. The diagnosis was bilateral bronchopneumonia and rheumatoid arthritis; or, possibly, acute rheumatic fever and rheumatic pneumonia.

Chest x-rays revealed irregular, patchy infiltrations throughout both lungs, chiefly the left, in keeping with acute bronchopneumonia; there was a pleural reaction at the bases. The heart was not enlarged. (Fig. 3A.) X-rays of the hands showed fusiform swelling of the interphalangeal



3A 3B

FIG. 3. (Case III) A, chest x-ray, March 12, 1954; irregular, patchy infiltrations in both lower lobes, more numerous on the left where there is confluence of densities in the mid-lung field; there is pleural reaction at bases. B, chest x-ray, May 13, 1954; fine, diffuse reticulations permeating both lungs, chiefly the right; pleural reaction at bases more evident.

joints. The joints themselves were not involved. Laboratory tests revealed an accelerated erythrocyte sedimentation rate, hemoglobin 79 per cent, red blood cells 4,220,000 per cu. mm., white blood cells 8,700 per cu. mm. with a normal differential; urine was negative; the Mazzini test was negative; serum albumin was 3.2 gm. per cent and serum globulin 3.2 gm. per cent; an electrocardiogram showed no abnormalities. The patient was treated with combined antibiotics, later with penicillin alone, also salicylates and sedatives. After one month of penicillin treatment the patient developed a generalized rash which gradually subsided. It was ascribed to penicillin sensitivity. Although the chest x-rays after three weeks showed considerable clearing of the lung fields, the rales persisted. The joint pains slowly abated, the fever declined and the patient was discharged on April 24, 1954.

Six days later the patient was admitted to the Morrisania City Hospital because of recurrence of fever, cough, chest and joint pains. The patient was acutely ill and dyspneic. The rash was still present over the chest and upper arms. The fingers, toes and knees were swollen and painful on motion. No abnormalities were found in the heart. On auscultation numerous rales were heard in both lungs, with pleural friction rub at the right base. The hemoglobin ranged between 8 and 11.4 gm. per cent, red blood cells 2,500,000 per cu. mm., white blood cells ranged between 5,800 and 10,000 per cu. mm. with a normal differential. The erythrocyte sedimentation rate was accelerated. The sputum was negative for acid fast bacilli. Mazzini test ±, Wassermann test 4+, urine was negative, serum albumin 2.6 gm. per cent, globulin 3.5 gm. per cent, cholesterol 97 mg. per cent, blood urea nitrogen 8.0 mg. per cent, fasting blood sugar 78 gm. per cent, cholesterol esters 165 gm. per cent, uric acid 1.5 mg. per cent, bilirubin 0.14 mg. per cent, thymol turbidity 15 units, cephalin flocculation 1+. Blood cultures were repeatedly negative for growth. Agglutination of the typhoid, paratyphoid group was negative. Chest x-rays now revealed fine diffuse interstitial striations throughout both lungs, especially the right, and less of the pneumonic exudate seen in previous films; a pleural reaction was present at the bases. (Fig. 3B.) The patient continued to have low-grade fever. On salicylate therapy the joint pains gradually subsided and the patient was discharged on May 26, 1954.

The patient was readmitted to the Morrisania City Hospital on July 1, 1954, because of recurrence of pain in the hands, wrists, shoulders and ankles, with swelling of the affected parts. The

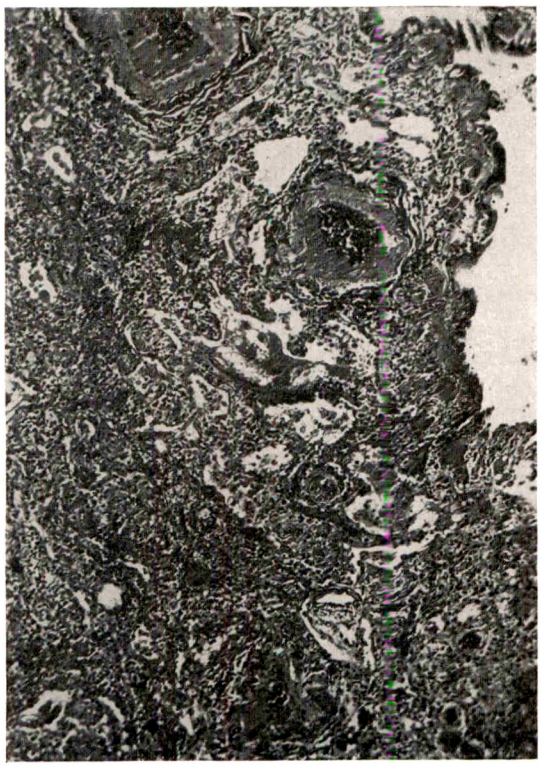


FIG. 3C. Lung biopsy (Case III) showing organizing and interstitial pneumonitis with patchy areas of fibrosis; several prominent blood vessels are seen.

patient was dyspneic and complained of a feeling of chest oppression. Physical and x-ray examinations revealed few changes from those noted previously. The laboratory findings were little changed. On July 2, 1954, a biopsy of the right lower lobe was done by Dr. M. Rubin. The excised specimen was examined by Dr. R. Lubliner. The findings were as follows: The lung was somewhat firmer than normal but fairly well aerated, the pleura slightly thickened. Cut section revealed pinkish-red parenchyma through which were scattered small strands of depressed, grayish tissue.

Microscopic examination showed marked alteration of normal architecture, foci of well aerated parenchyma alternating with foci of atelectasis. (Fig. 3C.) There was thickening of interalveolar septa due to increased vascularity, cellular infiltration and patchy fibrosis. In many instances there was hyaline thickening of the walls of the small vessels within the septa. The cellular infiltration was monocytic with a large number of plasma cells. There was marked hyperplasia of the alveolar lining cells. In other parts the alveolar spaces were not only distorted but elongated and narrowed due to the septal thickening and also to an organizing pneumonia. The alveolar spaces were filled with an admixture of fibrin, monocytes, alveolar macrophages and, in many instances, completely obliterated by the organized exudate. Both processes together caused scattered patches of pulmonary fibrosis; however, this in itself was not yet a prominent feature. There was slight fibrous thickening of the pleura and of the interlobular septa. There was moderate thickening of the interalveolar septa, also in well aerated areas. The diagnosis was interstitial and organizing pneumonitis with patchy pulmonary fibrosis.

The patient was treated with salicylates and sedation. The joint pains gradually subsided although the patient still had a low-grade fever at the time of her discharge to the Chest Clinic on July 23, 1954. A chest x-ray taken a week later showed slight decrease in the pulmonary markings. The pleural reaction was still present at both costophrenic sinuses. The patient still had painful and swollen knees and ankles and the fingers "felt tight" especially on arising.

PREVIOUSLY REPORTED CASES WITH AUTOPSIES

Ellman and Ball (1948)[7]: A forty-seven year old male had pleurisy progressing to empyema at eleven years of age. "Rheumatoid disease" appeared two and one-half years prior to present illness. Chest x-ray revealed fine reticulations throughout both lung fields with a chronic bronchopneumonia process. After admission to the hospital the patient developed cough, expectoration and, soon, thereafter, generalized purpura, pyrexia, dyspnea and cyanosis. The patient died three months after onset of the respiratory symptoms. Autopsy revealed dense pleural adhesions, interstitial fibrosis and pneumonitis with terminal bronchopneumonia, also some small abscesses; the blood vessels were largely normal.

Ellman and Ball (1948)[7]: A forty-eight year old female had recurring bronchitis. History revealed acute polyarthritis eight months prior to acute respiratory symptoms. Examination showed a rash over the arms and trunk. Chest x-ray showed bilateral basal opacities indicative of consolidation and marked reticular shadows extending into the mid-zones. The patient was treated with penicillin for suspected pneumonia without effect. She died one year after the onset of the "rheumatoid disease." Autopsy revealed

interstitial fibrosis and pneumonitis with terminal bronchopneumonia, also minute abscesses containing pus. The blood vessels showed no changes.

Katz and Auerbach (1951)[13]: A fifty-year old male with a history of asthma in childhood had increasing dyspnea, intermittent fever and weakness for about two years when he developed migratory polyarthritis and two months later cough and expectoration. Examination revealed advanced "rheumatoid disease" with demineralization of osseous structures, flexion and contracture deformities of hands, as well as muscular and subcutaneous atrophy of the parts. A chest x-ray four months after the appearance of the "rheumatoid disease" revealed diffuse, finely mottled and patchy nodular infiltrations throughout both lung fields. Later chest x-rays showed clearer definition of interstitial infiltrations. The patient showed iodide sensitivity when this drug was administered. He died in congestive heart failure. Autopsy revealed diffuse interstitial fibrosis of lungs with emphysema giving the organs a spongy appearance.

Bevans, Nadell, Demartini and Ragan (1954)[12]: The authors report the case of two patients with "malignant rheumatoid arthritis" whose fulminating course was characterized by widespread granulomatous lesions in the pleura, pericardium, heart, diaphragm, synovia, kidneys and lungs. In one patient the lungs at autopsy revealed compression of alveolar spaces but otherwise the parenchyma appeared normal. The other case, a forty-six year old female with "rheumatoid disease" of fifteen years' duration, developed pericarditis, pleuritis and eventually generalized anasarca. At autopsy several small yellow nodules were found in the left upper lobe which were soft and necrotic, also a solitary subpleural tubercle. Microscopically, the yellow nodules did not resemble tuberculosis, rather granulomas with necrotic centers. Fibrinoid material filled the alveolar spaces. Acid-fast and bacterial stains of these lesions were negative for organisms.

Christie (1954)[11]: A forty-seven year old male had rheumatoid symptoms since the age of thirty-five. Six days before the patient's first admission to the hospital he developed dyspnea, anorexia and edema of the legs. A right pleural effusion was aspirated. The patient was readmitted thirteen months later with cough and progressive edema. He died two months later. Autopsy showed fluid in both pleural cavities. The pleura was covered by fibrin. Several subpleural nodules were present but the intervening lung tissue was normal. Microscopic examination showed nodular lesions with spreading foci of fibrinous pneumonia undergoing central necrosis. At the edge of the necrotic foci the alveoli were filled with fibrin and the adjacent lung showed thickening of septa.

Christie (1954)[11]: A sixty-seven year old male developed rheumatoid symptoms and chest pain six months before admission to the hospital. At this time the patient had dyspnea, ankle edema and swollen joints. A chest x-ray showed cardiac enlargement, pulmonary congestion and fluid at the lung bases. Pleural aspiration revealed sterile fluid. The patient died twenty days after admission. Autopsy showed fibrous pleural adhesions, also fine fibrosis in the lung substance. Microscopic examination revealed radiating loose connective tissue. The scars appeared to be growing actively. The inner part of each was less cellular, with coarser collagen, whereas peripherally the collagen was fine and small round cells and fibroblasts were numerous. At the spreading edge the septa became thickened and encroached on the alveolar spaces and were gradually taken up in the scar.

Christie (1954)[11]: A fifty-three year old female had recurring seizures of pain which had the characteristics of Raynaud's syndrome. The presenting complaints were shortness of breath, cough and expectoration which had been present for three months. The patient revealed advanced rheumatoid disease of the joints. On a subsequent admission a right pleural friction rub was heard. The chest x-ray showed a round dense shadow 6 cm. in diameter in the right upper lobe. There was increasing emaciation and death. Autopsy revealed extensive adhesions obliterating the left pleural sac and also adhesions over the lateral aspect of the right lung. There was a firm rounded mass 7 cm. in diameter occupying the lower half of the right upper lobe. It was composed of pale, dry, friable tissue in which remnants of bronchi were identifiable. The mass was encapsulated by a layer of dense fibrous tissue. The remainder of the lung was normal except for old apical pleural scars. Microscopic examination revealed the massive lesion in the right upper lobe to be indistinguishable in structure from that of a subcutaneous periarticular nodule of rheumatoid arthritis. At the border of the scar the alveolar septa of the pulmonary tissue were thickened.

For the sake of completeness, Christie's lucid description of the pathology of the pulmonary lesions in "rheumatoid disease" is worth citing. This investigator notes that the pulmonary lesions in rheumatoid disease are as varied and widespread as those in other organs. They range from acute exudative foci to scars of loose or dense texture. In the cases studied, the pleura was found to be a prominent site but lesions were demonstrable in all parts of the lungs including the parenchyma, bronchi and lymphoid tissue. Christie describes the histologic appearances of the pulmonary lesions in "rheumatoid disease" as follows:[11] (1) proliferation of mononuclear cells in the alveolar septa with occasional binuclear formation in the early stages; (2) sharply localized areas of fibrinous pneumonia constituting a more severe grade of parenchymatous lesion. This might proceed to necrosis and softening or to organization; (3) connective tissue "buds," resembling those described by Masson and by Neubuerger and co-workers in rheumatic pneumonia, are sometimes present. These consist of minute nodules of connective tissue lying in alveolar ducts, budding from the wall; (4) focal scars of variable density in the pleura, alveolar walls, bronchi, vessels and peribronchial and perivascular connective tissue. Much of the fibrosis is non-specific although such areas usually contain more advanced foci recognizable as "rheumatoid" nodules; (5) focal scarring or diffuse fibrosis in the walls of the smaller pulmonary vessels and of the bronchial arteries; (6) acute fibrinous pleurisy and chronic pleural scarring independently of or in association with other pulmonary changes.

The autopsy findings in the cases of Ellman and Ball[7] showed evidence of advanced bronchopneumonic changes and small abscesses, in addition to diffuse interstitial fibrosis indicating that the disease was of considerable duration. The lung biopsy specimens reported in the present study were obtained relatively early in the course of the disease and consequently the diffuse interstitial pulmonary fibrosis could be established as the major feature of the disease. The vascular component in the two biopsy specimens was insignificant except in one patient who had received sulfonamide for ten days and then developed urticaria. An allergic element may have been a factor in the vascular changes although it was the impression of the pathologist that the alterations could be ascribed to the coexisting disease. Noteworthy in the case described by Bevans and co-workers[12] and in two by Christie[11] was the finding of localized granulomas in the lung and pleura which had the earmarks of "rheumatoid disease" found in joint lesions. Pleuritis was a feature in all the reported cases.

COMMENTS

A review of the cases reported in the literature and of those here recorded brings out a number of clinical features which, in addition to the pathologic changes previously described, would indicate that the pulmonary, like the joint lesions, of "rheumatoid disease" are part of the same disturbance. Visceral participation in "rheumatoid disease" is usually not recognized clinically; occasionally, it dominates the picture. The attention of the examiner is drawn to obvious articular lesions and, unless the visceral lesions had caused irreversible tissue damage or are demonstrable grossly, they are apt to be overlooked. In fact, this applies also to the postmortem findings. Christie[11] emphasized the fact that only significant active lesions are recognized at autopsy.

The histories of many of the patients indicate an allergic background. Each of the patients described in this paper had some evidence of pre-existing hypersensitivity in the form of urticaria of unknown cause or allergic reaction to sulfonamide or penicillin. The case reported by Katz and Auerbach[13] showed sensitivity to iodide. In the case reported by Bloom and J. H. Rubin[9] the fact that the pulmonary infiltrations appeared at the same time with the rheumatoid symptoms when the pleural fluid had resorbed was suggestive to the authors of an anaphylactic reaction. The same sequence occurred in Case II of the present series. Leys and Swift[8] suspected that the factor of heredity and allergy explained the "rheumatoid disease" in their patient, a boy of ten. Ellman and Ball[7] claim that the visceral and other lesions of "rheumatoid disease" may be explained by hypersensitivity manifestation in tissues. The factor of hypersensitivity in "rheumatoid disease" has been discussed fully in a number of publications by Rich and Gregory[16,17] and need not be reviewed here.

Of particular significance in the cases analyzed in this report was the time relationship between the appearance of symptoms and signs of the "rheumatoid disease" and pulmonary manifestations. In the vast majority the joint symptoms antedated the pulmonary manifestations. In a few the pulmonary symptoms appeared at the time of recrudescence of joint symptoms. On

rare occasion, as in Case III of the present series, acute joint and pulmonary manifestations appeared simultaneously at the onset of the disease. At times a nondescript "pneumonia" or "pleurisy" preceded the joint symptoms for a brief period and with the appearance of swollen, painful joints the pulmonary disease appeared to become reactivated. The later course of events was often characterized by a parallelism in the symptoms referable to both joint and lung lesions.

The chest x-ray findings in "rheumatoid disease" are of major interest since the lung changes portray dynamic phases in the evolution of the process. At times the pulmonary infiltrations are transitory.[8,9] The writer has not encountered any instance of remissions and exacerbations of pulmonary lesions demonstrable roentgenologically, but such occurrences may be expected if chest x-rays are taken frequently, especially during periods of recrudescence of the "rheumatoid disease." The most frequent expression of "rheumatoid disease" in the chest is as a pleuritis manifesting itself in small serous effusions; large collections of fluid are more apt to be associated with anasarca and to be caused by cardiac failure. The frequent elicitation of a pleural friction rub on auscultation during life and the almost inevitable presence of fibrinous or dense pleural adhesions in biopsy or autopsy specimens has been referred to previously.

The chest x-rays in "rheumatoid disease" usually show a bilateral, more or less symmetrical distribution of abnormal lung markings. The case reports, describing at autopsy circumscribed granulomatous lesions in the lungs or pleura, either do not include chest x-ray illustrations or only meager descriptions of the findings. The changes in the lungs are usually discovered accidentally at autopsy. One of the cases described by Christie[11] showed roentgenologically a round, dense shadow 6 cm. in diameter in the right upper lobe. Autopsy revealed it to be a large granuloma with histologic features similar to those found in periarticular nodules of "rheumatoid disease." Once it is recognized that rheumatoid lesions in the lungs may duplicate similar lesions in joints and other parts of the body, the finding of localized disease in chest x-rays will undoubtedly receive greater attention in the future.

The bilateral pulmonary mottlings seen roentgenologically in "rheumatoid disease," as shown in the two lung biopsy specimens and in the autopsy findings described previously, represent interstitial parenchymal changes related to the underlying disease. The infiltrations may be small and evenly distributed, simulating miliary tuberculosis,[9] or they may be of a nodular character affecting chiefly the mid and lower lung fields, simulating sarcoidosis, inhalational dust disease or bronchogenic tuberculosis,[7,8] or they may be irregular, of patchy distribution simulating viral or bacterial bronchopneumonia (Case III of present series). After subsidence of the acute stage of the disease, the markings assume a more linear configuration of a delicate or coarse reticular network such as one sees in lymphangitic carcinomatosis or diffuse interstitial fibrosis of the lungs (Cases I, II and III of present series). In long-standing disease secondary bronchopneumonia and suppuration tend to obscure the basic pattern. There is no characteristic picture characterizing the pulmonary lesions in "rheumatoid disease." But if one views the chest x-ray appearances in the light of the history, physical examination and laboratory findings, one may discern in the film "roentgenologic equivalents" in the sense that the gross and histologic findings offer "morphologic equivalents"[18] of similar conditions.

Other features of "rheumatoid disease" with special reference to pulmonary lesions will be described briefly under several subheadings.

Collagen Diseases and "Rheumatoid Disease." "Rheumatoid disease" is commonly included among the so-called collagen diseases, although some question such a relationship.[19] The collagen diseases constitute a group of disorders showing similar anatomic changes in the way of fibrinoid degeneration of connective tissue, also vascular and serous membrane involvements.[20,21] One of the reasons which has given rise to doubt that "rheumatoid disease" is a member of the collagen group is the fact that disseminated lupus erythematosus, scleroderma, dermatomyositis and polyarteritis nodosa, the more prominent members, are also often accompanied by arthritic, arthralgic and myositic symptoms. By the same token, however, it may be that in certain cases the joint involvement may constitute the major expression of the disease.

Support for the probable existence of a "common denominator"[21] between "rheumatoid disease" and the collagen group is obtained largely from indirect evidence. Cases have been described characterized by such blending of organ involvements,[22–24] including the presence of "rheumatoid disease," as to provide at least

an index of suspicion that the latter is a component member of the collagen group. The results of experimental[16,17] and histopathologic studies,[25–27] as well as the manner of response to hormonal treatment,[28,29] have shown parallel features in one way or another for the majority of the group. But, as emphasized repeatedly by Klemperer,[30] the collective term, "collagen diseases" includes a group of heterogeneous conditions which has in common a systemic alteration of connective tissue. This does not necessarily indicate a common cause. In fact, it would seem that the only incontestable link between the several members of the collagen group is that no cause is known.

"Rheumatoid Disease" and Rheumatic Pneumonia. Although the inclusion of rheumatic pneumonia in this discussion may not seem directly related to the subject, it may be pointed out that the pulmonary lesions of "rheumatoid disease" are not unlike those encountered in rheumatic pneumonia, including the frequent occurrence of pleurisy in both conditions. Some years ago Klinge[31] expressed the belief that rheumatoid arthritis and rheumatic fever are one and the same. Dawson[32] lent further support to this view by finding that the subcutaneous nodules in rheumatic fever and rheumatoid arthritis represent different phases of the same fundamental process. Young and Schwedel[33] found an extremely high incidence of rheumatic heart disease in rheumatoid arthritis, confirming previous pathologic studies.[4] Recently Kersley[34] analyzed the histories of 750 cases of rheumatoid arthritis and found thirty-eight in whom the onset of the disease was so acute as to resemble rheumatic fever. In 10 per cent of the entire group there was a past history of the latter.

Rheumatic pneumonia is an integral part of rheumatic fever, no less than the articular, cardiac and other visceral involvements. Rare instances have been described of patients succumbing in the acute stage of rheumatic fever as a result of pulmonary involvement without significant cardiac involvement.[35] There are also reports of patients who survived the pneumonia and shortly thereafter developed signs of rheumatic carditis. The writer had occasion to follow the course of a girl ten years of age whose pulmonary and cardiac disease progressed simultaneously and rapidly to a fatal issue within a period of seven months. Signs of rheumatic heart disease and chamber enlargement, as well as diffuse infiltrations in both lungs which could not be ascribed to cardiac decompensation, appeared to run a parallel course. Autopsy revealed evidence of advanced valvular heart disease and rheumatic pneumonia.[14]

Noteworthy is the fact that the lung changes in rheumatic pneumonia and virus pneumonia have many features in common, including the presence of a hilar membrane, mononuclear cell infiltration, non-bacterial exudate, capillary engorgement and interstitial edema. It is not improbable, as suspected by some observers, that rheumatic pneumonia may often be mistaken for a virus infection and that at least a small proportion of cases designated virus infection are instances of rheumatic pneumonia without outward manifestations.[35] Muirhead and Haley[37a] report the case of a twenty-five year old white woman who had known healed inactive cardiac lesions and a peculiar interstitial pneumonitis concomitantly. The pneumonitis was widespread, demonstrating a healed proliferative phase with extensive fibrosis and an active exudative phase. In addition, partly healed arterial lesions were observed in the lungs. The authors point out that this case offered an additional point of interest in that the pulmonary lesions resembled in certain respects the changes described by Hamman and Rich[38] in their cases of diffuse interstitial pulmonary fibrosis.

"Rheumatoid Disease" and Diffuse Interstitial Pulmonary Fibrosis. In certain cases of diffuse interstitial pulmonary fibrosis of unknown etiology the disease may be due to failure of resolution with resulting organization of an acute interstitial pneumonia of the type commonly known as primary atypical pneumonia.

Auerbach, Mims and Goodpasture[37b] point out that solution of fibrin has long been held to be a function of enzymes derived from polymorphonuclear leukocytes. Exudates which are rich in fibrin and poor in leukocytes are more apt to organize. These authors suggest that the widespread use of potent antimicrobial agents is contributing to a rising incidence of premature organization of pulmonary exudates with resulting fibrosis of lung tissue. An additional factor may be present in the allergic reactions which often accompany sulfonamide and penicillin treatment. Atypical pneumonias treated with antibiotics are not frequently associated with hypersensitivity reactions. The occasional encounter of diffuse interstitial fibrosis of the lungs in certain collagen, hyperergic and "rheumatoid" disease suggests a related mechanism.

Roentgenologic and pathologic changes in the lungs of such cases are indistinguishable from those found in patients with "rheumatoid disease," and in patients with interstitial pulmonary fibrosis of the type described by Hamman and Rich.[38] The latter draw attention to the fact that Winternitz examined the specimens obtained from their patients and found a striking resemblance to that found in the lungs of animals during experimental investigations on influenzal pneumonia. The lung biopsy specimens of the two cases discussed previously, as well as lung biopsy and autopsy material of a larger group of patients with diffuse interstitial pulmonary fibrosis,[15] were reviewed on various occasions by Zimmerman[39] and the latter expressed a similar opinion.

Hamman and Rich also draw attention in their publication to a group of cases of "current bronchopneumonia of unusual character and undetermined etiology" reported by Kneeland and Smetana[40] in which one of the cases corresponded clinically and anatomically with theirs. In the particular instance, a woman of forty-seven, developed urticaria simultaneously with chills and blood-streaked sputum. The patient was found to have progressive bronchopneumonia which was soon complicated by thrombophlebitis, anemia, hypoproteinemia and right-sided heart failure leading to death. At autopsy lesions were found in the pulmonary arteries suggestive of polyarteritis nodosa, in addition to diffuse interstitial pulmonary lesions characterized by a mononuclear cell exudate and a tendency to organization, also thickening of alveolar septa and narrowing of alveolar spaces. The patient had received sulfapyridine and the vascular changes may have been related to the drug.

Of particular significance is the fact that among the fifty-two patients with atypical bronchopneumonia described by Kneeland and Smetana[40] was a group of six "in whom the clinical picture of a severe and prolonged bronchopneumonia was complicated by a variety of phenomena suggesting that tissues other than pulmonary alone were involved. Among these phenomena were migratory polyarthritis, erythematous skin eruptions, slight enlargement of the spleen and lymph glands, jaundice, gross hematuria, fibrinous pericarditis and encephalitis. All six happened to be females and as these perplexing clinical panoramas unfolded themselves the notion kept recurring that there must be an associated element of diffuse vascular disease of the type seen in disseminated lupus or periarteritis nodosa."

ADDENDUM

Since submitting this article, I have followed the course of a fourth patient with "rheumatoid disease" and diffuse interstitial pulmonary fibrosis. A sixty-five year old woman was admitted to the Medical Division of Montefiore Hospital (Dr. Louis Leiter, Chief) with a history of concurrent onset of joint pains, fever and acute respiratory symptoms diagnosed as pneumonia in another hospital. The disease was of eight months' duration. The chest x-rays and lung biopsy specimen revealed a picture similar to that noted in the cases cited. Noteworthy was the fact that in this case the fine rales heard throughout both lungs, which were ascribed to fibrinous pleurisy, probably originated in the alveolar spaces since the small bronchioles showed no histologic abnormalities while the alveolar spaces showed focal atelectasis. The blood vessels were unremarkable. The specimen showed only minimal thickening of the visceral pleura.

SUMMARY

"Rheumatoid disease" is a more descriptive term than rheumatoid arthritis since the condition affects various parts of the body including the locomotor system. The disease may be associated with pulmonary lesions which are part of the same disturbance. The pulmonary lesions may simulate acute atypical pneumonia, acute rheumatic pneumonia or acute, diffuse interstitial pulmonary fibrosis. In some cases these several manifestations represent one and the same disease.

Acknowledgments: I wish to thank Dr. Harry M. Zimmerman, Chief of the Laboratory Division of Montefiore Hospital for his help and criticism in the preparation of this paper; Dr. Morris Rubin of the Thoracic Surgical Department for the performance of the lung biopsies and Drs. J. Hasson and Ruth Lubliner of the Laboratory Division for their interpretation of the findings of the biopsy specimens.

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Liver Disease in Sickle Cell Anemia*

A Correlation of Clinical, Biochemical, Histologic and Histochemical Observations

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Relatively little has been published regarding liver function in patients with sickle cell anemia.[1] Clinical descriptions occasionally refer to the occurrence of some form of "hepatitis" or "evidence of increasing hepatocellular damage" in this disease.[2,3] In many instances chronic enlargement of the liver has been noted and transient hepatomegaly often occurs during crises. The frequent findings of bilirubinuria and high values for direct-reacting serum bilirubin[4] suggest that hepatic involvement is common. In recent years clinical and laboratory evidence of liver dysfunction in patients with sickle cell anemia has been attributed to viral hepatitis, particularly in those who have received many blood transfusions.[5]

In at least twelve of fifteen patients with sickle cell anemia, Fenichel, Watson and Eirich[6] noted non-specific abnormalities of the electrophoretic pattern for serum proteins similar to those which occur in viral hepatitis and portal cirrhosis. The observations of these authors as well as the findings[7] in two of our patients with sickle cell anemia suggested that at least some of the hepatic dysfunction in this disease might be a manifestation of the sickle cell anemia itself. Therefore, further investigations were undertaken in an attempt to determine the nature of the liver changes associated with this disease. In addition to clinical, biochemical and electrophoretic studies, histologic and histochemical observations were made on liver tissue obtained by needle biopsy from four patients. After these investigations had been started an excellent report on the clinical and biochemical findings in fifty patients and autopsy studies of twenty-one was published by Green, Conley and Berthrong.[8] They concluded that the hepatic disease present in some of their cases seemed to be a specific manifestation of sickle cell anemia and could not be explained on any other basis.

METHODS

Biochemical Determinations. The following biochemical determinations were made: total and direct-reacting serum bilirubins,[9] blood glucose,[10] serum alkaline phosphatase,[11] cephalin-cholesterol flocculation test,[12,13] thymol turbidity test,[14] thymol flocculation test,[15] serum colloidal gold reaction[16] (Boerner reagent[17]), scarlet red test,[18] serum albumin and globulins and total serum proteins,[19] serum amylase[20] and lipase,[21] urine bilirubin[22] and urobilinogen.[23,24] Bromsulfalein retention[25] was also determined. For two patients duplicate determinations of serum fatty acids,[26] phospholipids[27] and total and free serum cholesterol[28] were made in the Research Laboratory, Jefferson Medical College, under the direction of Dr. I. J. Pincus. The cholesterol ester fraction noted in Table I was calculated by subtracting the free from the total cholesterol value. The remaining serum lipid determinations were made at the Graduate Hospital. These included total serum lipids[29] and total serum cholesterol and esterified fraction.[30] The free cholesterol was calculated as the difference between the total and ester values.

As indicated in Table VI, most biochemical determinations for patient no. 4 were made at the Philadelphia General Hospital. These included

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TABLE I

CASE I—LABORATORY DATA*

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct	Total	Bile	Urobilinogen	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu.mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg. %	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr.	500–800 mg. %	9–10 mg. % P	7.2–16.2 mEq./L.	150–280 mg. %	50–80% of total	50–70% of total	Up to .5	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose–5 mg./kg. body wt. Normal less than 5% in 45 min.
11/30/52	8.5	....	....	36.6	....	....	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/1/52	....	....	....	....	9.6	15.2	...	.....	...	..	..	248	128	120	.52	7.60 3.98/3.62	3+	3+	4.0	3+	0	0	15.8	...
12/2/52	7.4	....	....	....	....	....	...	>12.2	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	...	
12/3/52	6.5	2.5	24.3	36.4	....	....	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/4/52	....	....	....	28.0	....	....	...	.>5.1	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/5/52	....	....	....	28.0	15.2	19.0	...	.....	...	..	..	...	...	...	...	6.88 3.45/3.43	0	0	4.0	0	0	0	17.2	...
12/6/52	13.0	4.4	....	26.2	....	....	...	.....	...	..	..	186	116	70	.62	.....	...	...	...	...	..	..	....	...
12/8/52	10.0	....	....	21.4	8.9	13.2	...	.....	...	..	..	160	70	90	.44	6.40 3.52/2.88	±	±	3.0	0	0	0	12.0	...
12/9/52	9.3	3.3	....	16.5	....	....	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/11/52	11.9	4.0	....	17.4	....	....	...	>5.2	...	...	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/13/52	10.2	....	4.8	15.6	20.8	26.4	...	.....	...	..	..	150	90	60	.60	.....	...	...	...	...	..	..	8.0	...
12/15/52	14.4	4.75	....	12.5	....	....	...	1.3	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	.	...
12/19/52	....	....	....	....	....	....	...	.....	...	..	..	...	...	...	...	.....	2+	2+	6.0	2+	0	0	.	...
12/21/52	....	....	....	....	19.0	24.5	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/22/52	13.0	4.8	....	10.0	....	....	3+	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...

TABLE 1 (*Continued*)

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct	Total	Bile	Urobilinogen	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu.mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg. %	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr.	500–800 mg. %	9–10 mg. % P	7.2–16.2 mEq./L.	150–230 mg. %	50–30% of total	50–70% of total	Up to .5	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose–5 mg./kg. body wt. Normal less than 5% in 45 min.
12/26/52	11.4	4.0	.3	13.0	....	....	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/27/52	....	....	....	....	14.6	18.2	...	.....	800	..	..	226	132	94	.59	8.40 4.52/3.88	...	0	3.0	0	0	0	14.0	...
12/29/52	....	....	....	....	....	....	...	.....	...	..	..	...	...	...	...	.....	0	0	3.0	0	0	0	....	...
12/30/52	13.4	4.4	1.2	21.8	....	....	...	1.4	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
12/31/52	....	....	....	....	....	....	...	.....	656	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
1/7/53	12.2	4.4	1.2	13.4	5.3	6.9	...	.....	...	..	..	196	90	106	.46	.....	...	...	...	...	..	..	8.2	...
1/14/53	11.5	4.1	1.2	13.0	3.6	4.8	...	.3	...	...	..	210	110	01	.01	7.36 2.00/0.01	0	0	2.5	0	0	0	....	5.8
1/21/53	....	....	....	....	....	....	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	9.8	...
1/22/53	....	....	....	....	2.2	3.2	...	.....	...	..	..	140	72	68	.51	.....	...	...	...	...	..	..	8.6	...
1/27/53	....	....	....	....	2.1	2.9	...	.....	...	..	..	264	124	140	.47	6.40 4.12/2.28	...	...	...	...	..	..	9.0	...
1/31/53	....	....	....	....	....	....	...	.....	...	..	..	264	134	130	.51	.....	...	...	...	...	..	..	....	...
2/3/53	....	....	....	....	1.9	2.5	...	.....	...	..	..	...	...	...	...	.....	...	...	...	...	..	..	....	...
2/4/53	....	....	....	....	1.7	2.6	0	1.8	...	..	..	260	140	120	.54	6.96 3.98/2.98	0	0	2.0	0	0	0	7.4	...
2/5/53	12.8	4.5	....	12.0	....	....	...	.....	...	..	..	...	...	...	...	.....	0	0	1.5	0	0	0	....	...

* Abbreviations—R.B.C.—red blood cells; Retic.—reticulocytes; W.B.C.—white blood cells; CCF—cephalin cholesterol flocculation; TT—thymol turbidity; TF—thymol flocculation; SCG—scarlet colloidal gold; SR—scarlet red.

total and direct-reacting serum bilirubins,[9] total and free serum cholesterol,[31] total serum proteins, albumin and globulins,[32] cephalin-cholesterol flocculation,[12] thymol turbidity[13] and thymol flocculation[14] tests, serum alkaline phosphatase[33] and bromsulphalein retention.[25] Urine bilirubin[24] and twenty-four-hour urinary urobilinogen excretion[34] were determined.

Serum proteins were studied by paper electrophoresis.[35] Electrophoretic analyses of three samples of serum and one of plasma (with heparin added as anticoagulant) were carried out in an Aminco-Stern universal electrophoresis apparatus.* The samples were diluted with barbiturate buffer of ionic strength, 0.1 and pH 8.45 at 21°c. The determinations were made at 2°c. in the standard cell having a cross sectional area of 0.75 sq. cm. The optical system included a cylindrical lens.

Histologic and Histochemical Methods. The technic of liver biopsy and the histologic and histochemical methods employed during this investigation have already been described in detail.[7] The liver tissue obtained by needle biopsy was aspirated into cobalt-formal-calcium solution and fixed in either this or 80 per cent ethanol. Frozen and paraffin sections were prepared as required. For histologic studies the following staining methods were employed: hematoxylin and eosin, a modification of the Mallory stain for connective tissues and a silver technic for reticulin. Histochemical procedures included Oil Red 0 and Sudan Black B for lipids in general, Nile Blue sulfate for the differentiation of acidic and neutral lipids, the acid hematein test for phospholipids and phosphatidic acids, the periodic acid-Schiff's reaction with amylase-treated controls for glycogen, the Feulgen reaction and pyronin-methyl green staining with appropriate enzyme-treated controls for desoxypentose and pentose nucleic acids, respectively, and a modification of Perls' test for iron-containing pigments.

CASE REPORTS

Case 1. R. G., a twenty-one year old Negress, was admitted to the Graduate Hospital, November 30, 1952, because of lower abdominal pain and jaundice. She stated that on May 4, 1952, she gave birth to a normal child and that because of anemia she had received two blood transfusions before delivery and one after it. About two months later she first noticed that her eyes were jaundiced. There was no associated pain, anorexia, fever or pruritus. Her urine became dark and her stools were brown. The icterus increased progressively; however, she felt well enough to continue her housework. During September, 1952, she began to experience sharp, shooting pains in her arms and legs, across her lower abdomen just above the symphysis pubis and across her lower back. This intermittent pain persisted for about one week and then subsided spontaneously. During the night before admission it returned with greater intensity than previously and was more marked in the lower extremities. This episode persisted for approximately twenty-four hours. Associated with it were malaise, generalized weakness and once, vomiting.

Every few months since childhood she had experienced episodes of sharp, shooting pains in her arms and legs. She had always been thin, felt weak and, compared with her friends, had undue shortness of breath on exertion. There was no history of exposure to hepatotoxic or hemotoxic drugs or of previous jaundice. She drank beer only occasionally. There was no family history of anemia, jaundice or vague chronic illness.

She was 5 feet 4 inches tall, and weighed 110 pounds. Her sclerae were deeply icteric. The retinal veins were tortuous. The heart was normal in size. The blood pressure was 122/65 mm. Hg. Her liver was palpable 2 cm. below the costal margin in the mid-clavicular line on inspiration. Its edge was round and not tender. There were no other relevant, abnormal physical findings.

Her hemoglobin concentration was 8.5 mg. per 100 ml. blood, white cell count 36,600 per cu. mm. with 84 per cent neutrophils. Peripheral blood smear showed many sickle and target cells and 35 normoblasts per 100 white blood cells. Anisocytosis, poikilocytosis, polychromatophilia and Howell-Jolly bodies were noted. A sternal bone marrow smear showed a marked increase of cells, especially normoblasts and pronormoblasts, and a moderate shift to the left of granular leukocytes. A diagnosis of sickle cell anemia in crisis was made. The sickling test was subsequently found positive.

The day following admission only slight arm pain persisted. After this subsided there was no further recurrence of pain. Although malaise

* (Model 5-8000 of the American Instrument Co., Silver Spring, Md.)

and weakness were prominent for the next three days, she was comfortable and her appetite increased slightly. She received a total of 3,500 ml. of blood between December 3rd and December 14th. Her temperature which was 101°F. on November 30th, rose to 105°F. on December 1st, fell to 102°F. for two days and remained elevated at about 100–101°F. until December 13th, following which, with the exception of an occasional rise to 99.6°F., it remained normal.

Because the reticulocyte count had decreased to 4.8 per cent on December 13th and was only 3 per cent nine days later, it was considered that the hemolytic process had largely subsided. She felt much better and ate more of the highly nutritious diet prescribed. Deep icterus persisted. The sedimentation rate (Wintrobe) on December 3rd was 13 mm. per hour. The blood Wassermann and Coombs tests were negative. One and two plus albuminuria accompanied the hemolytic crisis.

The presence of a hyperplastic bone marrow and of marked reticulocytosis in a patient with sickle cell anemia permitted the diagnosis of a hemolytic type of jaundice with certainty. However, it was impossible to attribute the icterus solely to the hemolytic process. The level of serum bilirubin, particularly of the direct-reacting fraction, and the presence of bile in the urine caused consideration to be given to another pathogenetic mechanism for the jaundice. Choledocholithiasis was the only extrahepatic cause given serious consideration. Her recent pregnancy, the known high incidence of cholelithiasis in sickle cell anemia, the high levels of serum alkaline phosphatase and the normal flocculation tests observed during certain phases of her illness lent some support to this hypothesis. An associated intrahepatic rather than posthepatic icterus was favored by such features as the absence of abdominal pain at the onset of icterus, the history of blood transfusions two months before (suggesting viral hepatitis), the absence of pain referable to the biliary tract at any time during her illness and the abnormal cephalin-cholesterol flocculation test on admission. In spite of the high incidence of gallstones in sickle cell anemia, no report of jaundice due to calculous obstruction could be found in the literature. However, Henderson[2] and Green, Conley and Berthrong[8] have each reported one case in which stones were demonstrated in the common duct at autopsy.

The consensus clinically favoured hepatocellular disease rather than extrahepatic obstruction occurring in association with the hemolytic process of the sickle cell anemia crisis.

On December 27th her liver was just palpable at the costal margin on deep inspiration. A needle biopsy was taken at 9:30 A.M. after she had been fasting for twelve hours. Venous blood was withdrawn between 9:05 and 9:20 A.M. The results of some of the laboratory determinations on this specimen and of other studies throughout hospitalization are recorded in Table I. The blood sugar concentration was 99 mg. per cent and the serum amylase and lipase values were 109 mg. per cent and 0.8 ml. N/20 NaOH, respectively. Electrophoretic analysis (Table II) of the plasma revealed that the amount of fibrinogen was reduced. It was probably even less than the value given in the table because the pattern was difficult to interpret in the fibrinogen region. There was a reduction in albumin and an increase in gamma globulin in both serum and plasma.

Microscopic examination of the biopsy specimen (Figs. 1 and 2) revealed disruption of normal liver architecture. There was diffuse, parenchymal cell degeneration, which was more marked in some regions, and areas of necrosis, which varied in size, showed no special zonal distribution and were only sometimes associated with mononuclear cell infiltration. The supporting reticulin of the necrotic areas had collapsed. There was slight mononuclear cell infiltration of portal areas. Bile pigment was found in the parenchymal cells and especially where degeneration and necrosis were prominent. Canalicular bile plugs were common, especially centrilobularly. In addition to the areas of necrosis, focal areas of "atrophy" were also found. The sinusoids were not widened. In some sites ghosts of red blood cells were present in clumps. Kupffer cells were prominent and filled with bile pigment.

She continued to improve and the icterus diminished considerably. On January 21, 1953, cholecystography revealed a cluster of radiopacities of calcific density which were considered to represent biliary calculi in a nonfunctioning gallbladder. There were no radiologic abnormalities in her chest, skull or long bones. The electrocardiogram was compatible with left ventricular hypertrophy.

On February 4th a second liver biopsy was

taken after fifteen hours' fasting. The results of laboratory determinations made on blood drawn five hours before the biopsy are noted in Table I. The serum amylase and lipase values were 126 mg. per cent and 0.7 ml. N/20 NaOH, respectively. She was discharged the day following the biopsy, feeling well but slightly icteric.

TABLE II

RESULTS OF ELECTROPHORETIC ANALYSIS OF SERUM AND PLASMA SAMPLES

Case	Sample and Boundary	Potential Gradient	Per cent of Total Protein						A/G Ratio	Mobility
			Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	Fibrinogen	γ-Globulin		
		v/cm.								
I	Serum: Ascdg.	6.21	45.2	4.14	7.96	15.9	.....	26.7	0.81	7.09
	Desdg.		42.3	5.38	8.46	15.0	.....	28.8	0.82	6.44
	Plasma: Ascdg.	6.49	43.2	(19.8)[1]		11.2	8.2	17.7	.....	6.68
II	Serum: Ascdg.	6.54	32.1	10.51	15.15	3.67	("X" 12.85)[2]	26.9	0.47	6.64
	Desdg.		36.1	7.33	15.2	7.33	("X" 14.15)[2]	19.9	0.56	6.36
III	Serum: Ascdg.	6.49	47.1	5.14	7.5	9.09	("X" 8.7)[2] ("Y" 9.09)[3]	13.05	0.89	6.75
	Desdg.		44.9	5.88	4.2	8.4	("X" 6.73)[2] ("Y" 10.05)[3]	19.03	0.82	6.47
Normal Values										
Serum[4]			56.8 ± 3	7.2 ± 1.2	8.7 ± 1.5	12.8 ± 2.3	.....	14.4 ± 2.4	1.33 ± 0.18	.....
Plasma[5]			59.6	6.7	8.8	11.0	4.8	9.1	1.68	.....

[1] Value is for α_1- and α_2-globulins together.
[2] "X" represents a component having a mobility intermediate between α_2- and β-globulins.
[3] "Y" represents a component having a mobility intermediate between β- and γ-globulins and may be fibrinogen.
[4] Values are from Reiner, Fenichel and Stern (1950).
[5] Values are from Deutsch and Goodloe (1945).

The lobular architecture was normal in the second biopsy specimen. (Figs. 3 and 4.) The outstanding change was the persistence of several focal lesions of parenchymal cells which had undergone degeneration and disintegration and had pyknotic nuclei. In most of these areas there were small collections of lymphocytes and monocytes. There were other small focal lesions, often mid-zonal, in which there was a "falling out" of cells. There was no portal inflammation. The sinusoids were dilated but no engorgement or clumping of red blood cells was present. Kupffer cells were prominent and contained moderate amounts of iron-containing pigment. There was no increase in connective tissue. The arteries were normal.

The histochemical observations for both biopsy specimens are summarized in Table III. Lipid studies were not made on the first one. Minimal amounts of acidic lipids, however, were found in the second specimen. The cytoplasm of the parenchymal cells was well filled with glycogen in the first but not in the second one. Nuclear glycogen vacuolation was absent. In both specimens the amounts of nuclear desoxypentose nucleic acids but not cytoplasmic pentose nucleic acids appeared to be reduced. Small amounts of hemosiderin were found in the parenchymal cells, especially degenerated ones, and larger amounts in the Kupffer cells.

There were certain clinical features which made it difficult to accept a diagnosis of viral hepatitis. Her icterus had persisted over a period of five months prior to hospitalization. The episodes of pain experienced two months before admission could have been associated with crises of sickle cell anemia and the pain which led to hospitalization most certainly was. Icterus persisted for two months in the hospital, making a total period of seven months of jaundice and, at the time of her discharge from hospital, her serum bilirubin was still elevated. The first liver biopsy findings revealed hepatocellular damage evidenced by areas of parenchymal cell degeneration and necrotic ones which were predominantly focal. These histologic findings are not specific for any disease. The evidences of bile retention, especially bile thrombi in canaliculi, are compatible with obstruction, either extra- or intrahepatic, or parenchymal disease. In the second biopsy, taken about six weeks after the first, focal

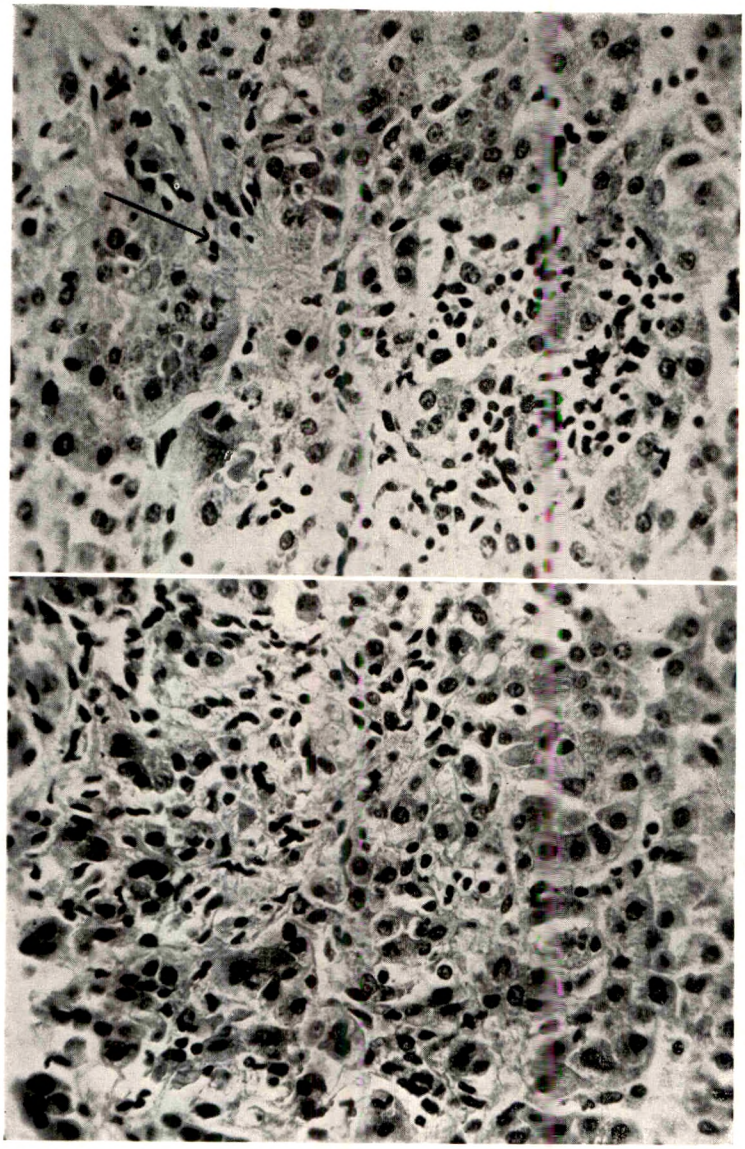


FIG. 1. Patient No. 1; first liver biopsy. At one end of a venous channel there is a small area of parenchymal cell necrosis (arrow). Adjacent to it is a larger area of parenchymal cell necrosis and degeneration associated with mononuclear cell infiltration. Canalicular bile plugs are seen; paraffin section (7 micra); hematoxylin and eosin; × 260.

FIG. 2. Patient No. 1; first liver biopsy showing an area of parenchymal cell necrosis and degeneration with adjacent liver cord disorganization. Canalicular bile plugs are prominent. Paraffin section (7 micra); hematoxylin and eosin; × 260.

parenchymal lesions persisted. If this patient had viral hepatitis associated with her hemolytic jaundice, it would be difficult to know when during her seven-month period of jaundice it played a part unless one is willing to accept that it was present throughout the entire course of her illness. Although impossible to exclude viral hepatitis, it was felt that the hepatic changes were due to some other cause. Drugs, bacterial infections or other agents could not be incriminated. It was considered, therefore, that the hepatic disease might be causally related to the sickle cell anemia itself.

CASE II. R. B., a forty year old Negro was

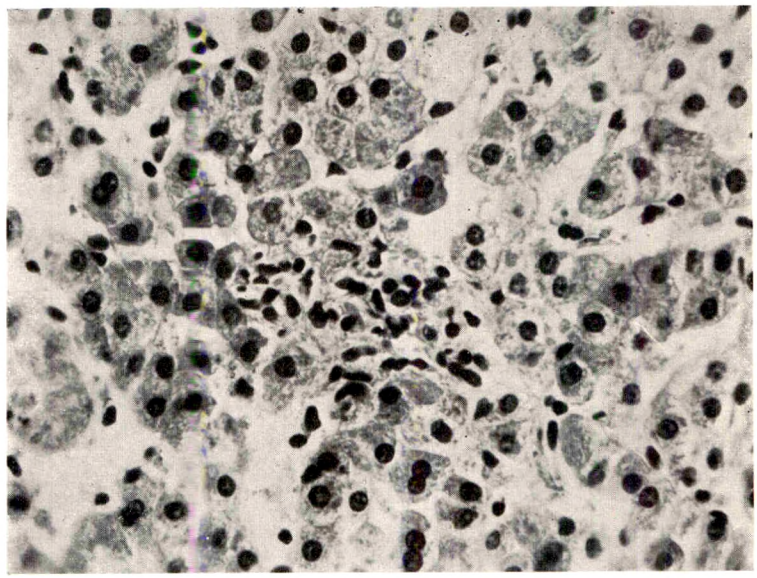


FIG. 3. Patient No. 1; second liver biopsy. A focal lesion consisting of degenerating and necrotic parenchymal cells with mononuclear cell infiltration. There is dilatation of sinusoids and Kupffer cell prominence. Paraffin section (7 micra); hematoxylin and eosin; × 260.

TABLE III

LIVER BIOPSIES—HISTOCHEMICAL OBSERVATIONS[1]

Case		Lipids[2]		Glycogen[3]		Nucleic Acids[4]		Iron Pigment	
		Cytoplasmic Droplets	Cysts	Cytoplasmic	Nuclear (%)	Nucl. D.N.A.	Cyto. P.N.A.	Parenchymal Cells	Kupffer Cells
I	12/27/52	. . . . .	. .	4 unif.	0	1	4	1	3
	2/4/53	*tr.*, v. sm., occas. lg., ac., centr.	0	2½ unif.	0	1	3	*tr.*	3
II		*v.f. tr.*, sm., neut., scat.	0	3½ unif.	0–20	1	3	3	3
III*		(0)	(0)	(3)	(0)	(1)	(3)	(4)	(3)
IV		0	0	3 unif.	1	. .	3	>4	>4

[1] Grading of observations for lipids, glycogen, nucleic acids and iron pigment, ranges from 0 to 4 and includes within the interval 0–1, v.f. tr. (very faint trace), f. tr. (faint trace) and tr. (trace).

[2] Lipids: Separate data are given for cytoplasmic fat droplets and for fatty cysts as follows:
Size: v.sm.—very small; sm.—small; lg.—large
Reaction: ac.—acidic; neut.—neutral
Distribution within hepatic lobules: centr.—central; scat.—scattered

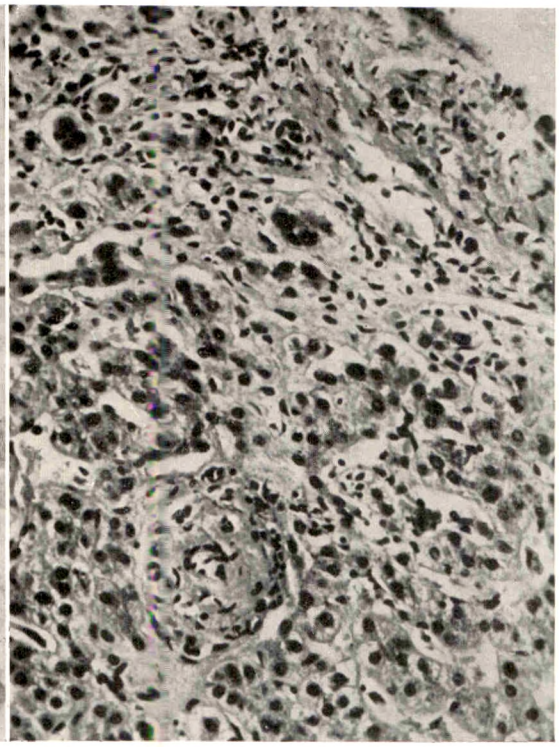
[3] Glycogen: Cytoplasmic: Distribution: unif.—uniform; Nuclear: Percentage of nuclei containing some glycogen.

[4] Nucl. D.N.A.—Nuclear desoxypentose nucleic acid.
Cyto. P.N.A.—Cytoplasmic pentose nucleic acid.

* Little tissue present making the histochemical observations, in parentheses, of limited value.



4



5

FIG. 4. Patient No. 2; first liver biopsy showing clumps of erythrocytes in widened sinusoids. Some of the red cell clumps are bordered by nuclei of Kupffer cells which are prominent. Paraffin section (7 micra); hematoxylin and eosin; × 420.

FIG. 5. Patient No. 2; second liver biopsy. There are bands of cellular connective tissue and adjacent degenerated parenchymal cells. An arteriole with marked narrowing of the lumen is seen in the left lower corner. Paraffin section (7 micra); hematoxylin and eosin; × 110.

admitted to the Graduate Hospital on March 1, 1948, because of episodes of dull, non-radiating, mid-epigastric pain lasting two to three days a week, of one year's duration. The pain usually occurred one or two hours after breakfast and the noon meal, and was associated with nausea, vomiting and generalized weakness. There were no alleviating or aggravating factors. In September, 1947, the pain increased in frequency until it occurred daily. Nausea and vomiting disappeared. He had noticed intermittent scleral icterus since 1946 and it had become constant three or four months prior to admission. There was no fever or pruritus. His stools were brown and, since June, 1947, the urine had been darker than previously. Despite a good appetite, he had lost 20 pounds in weight during the year before admission. No other abnormalities were elicited on systemic review. There was no history of alcoholism.

Examination revealed a 5 foot 6 inch tall, moderately well nourished, gracile male. His sclerae were icteric. The retinal veins were quite tortuous. The heart was enlarged to the anterior axillary line in the sixth interspace. There was a grade 3 systolic murmur at the apex. The blood pressure was 120/80 mm. Hg. His liver was palpable in the mid-clavicular line about four finger breadths below the right costal margin. There was a small superficial pretibial ulcer on his left leg. No other abnormalities on physical examination were found.

The hemoglobin was 8.0 gm., the red cell count was 2,040,000 per cu. mm., reticulocytes 2.6 per cent, white cell count 8,300 per cu. mm. Sickling, polychromatophilia and nucleated erythrocytes were noted in the smear of peripheral blood. A diagnosis of sickle cell anemia was made. There were many sickled erythrocytes and markedly active normoblastic erythropoiesis in the bone marrow. The direct Van den Bergh reaction was 1.68 mg. per cent and the

total, 3.3 mg. per cent. The blood Wassermann was negative, serum amylase 30 mg. per cent and serum lipase, 0.1 ml. N/20 NaOH. The results of other laboratory tests on this and subsequent admissions are given in Table IV. Radio-opacities 2 mm. in diameter were scattered throughout both lung fields; there was a moderate degree of left ventricular enlargement and slight prominence of the pulmonary conus sector. The trabecular pattern of the vertebral bodies was coarser than normal. A markedly deformed duodenal cap with a constant fleck in the mid-portion was interpreted as an active ulcer. Cholecystography and x-rays of the skull and long bones were normal. The electrocardiogram showed non-specific T wave changes. Gastric analysis following an Ewald test meal showed 22 units free acid at seventy-five minutes.

On March 3, 1948, chills and fever to 104.8°F. followed the administration of 500 ml. of blood. Following medical management, the duodenal ulcer healed radiologically. He was discharged on April 19, 1948.

On February 27, 1950, he was readmitted for blood transfusions. He had had only a mild exacerbation of epigastric pain in June, 1949. The findings on examination were similar to those on the previous admission except that his liver could not be palpated in spite of radiologic evidence of moderate hepatomegaly. The blood pressure was 112/70 mm. Hg. Seven 500-ml. blood transfusions were given. The PPD test no. 1 was positive and the histoplasmin test negative. He was given a bland diet. A liver biopsy was taken after he had been fasting for approximately twelve hours. He was discharged on March 15, 1950.

Some of the histologic findings are illustrated in Figure 4. There was marked sinusoidal widening which caused the liver cords to appear prominent. There were clumps of erythrocytes in sinusoids and some were engulfed in Kupffer cells. Except for very occasional, small focal lesions in which liver cells appeared to be atrophic or to have "fallen out," the parenchymal cells were normal. There was slight periarterial thickening. Very little pigment, probably hemosiderin, was present in Kupffer cells and in some parenchymal cells. However, no studies with special stains were made. Some nuclear "ballooning," suggestive of glycogen vacuolation, was present. There was no increase in fibrous tissue.

On February 2, 1953, he was readmitted for blood transfusions. Since discharge in March, 1950, he received a total of 8,000 ml. of blood because of anemia. There had been one recurrence of duodenal ulcer in the interim. On one occasion the liver could no longer be palpated. On this admission he was asymptomatic and weighed 120 pounds (17 per cent underweight). The blood pressure was 124/78 mm. Hg. The liver was grossly enlarged, extending to 10 cm. below the right costal margin in the mid-clavicular line and 8 cm. below the level of the xiphoid. Its edge was round, firm and not tender. Its surface was smooth. Physical findings were otherwise unchanged. A coarsened trabecular pattern at the distal end of the humerus and at the proximal end of the ulna were demonstrated radiologically. He received the routine ward diet. On February 5th a needle biopsy of his liver was taken at 10:40 A.M. after he had been fasting for approximately twelve hours. Venous blood was withdrawn at 9:36–10:40 A.M. The results of the biochemical studies are recorded in Table IV. Other determinations performed on the same blood sample included serum amylase and lipase which were 89 mg. per cent and 0.4 ml. N/20 NaOH, respectively. Qualitative paper electrophoretic studies of the serum from this patient did not reveal any abnormalities; however, electrophoretic analysis showed a reduction in the albumin and beta-globulins. (Table II.) A new component ("X"), between the $alpha_2$- and beta-globulins, was present in considerable amount. He was discharged on February 12, 1953.

Microscopic examination of the biopsy specimen revealed several interesting findings. (Figs. 5 and 6.) There were cellular connective tissue scars in areas without distinct distribution and an increase in periportal connective tissue. The parenchymal cells adjacent to the fibrous tissue were degenerated. The cytoplasm of many parenchymal cells appeared coarsely granular and some contained bile pigment. As shown in Figure 6, there were some focal areas of degenerated hemosiderin laden parenchymal cells. There were also occasional small focal areas of liver cell degeneration and focal areas where liver cells appeared to be undergoing resorption. The walls of some of the arteries were thickened and this change involved adventitial and intimal regions. The lumen of one vessel shown in Figure 5 was considerably narrowed. The sinusoids were dilated and contained some clumps of red

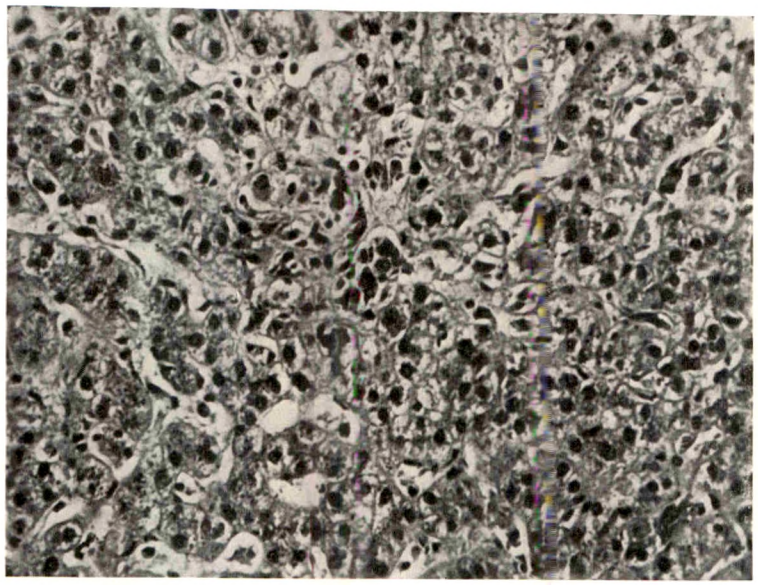


FIG. 6. Patient No. 2; second liver biopsy. In the center of the photograph is a small focal area of degenerated hemosiderin-laden parenchymal cells. The remainder show marked granularity due to hemosiderin. Paraffin section (7 micra); hematoxylin and eosin; × 110.

blood cells. The Kupffer cells were more prominent than in the first specimen and were filled with iron-containing pigment. Occasionally, they bordered the red blood cell clumps. The most significant change was the slight but definite presence of portal cirrhosis.

The histochemical observations on the second biopsy specimen are recorded in Table III. Very faint traces of neutral lipids were found in some of the parenchymal cells all of which contained large amounts of glycogen. Considerable variations in the incidence of nuclear glycogen vacuolation was noted. There might have been some reduction in the amount of nuclear desoxypentose nucleic acids but not in the cytoplasmic pentose nucleic acids. There were moderate amounts of hemosiderin in both the parenchymal and Kupffer cells but not in connective tissue scars. Some bile pigments were present in parenchymal cells.

CASE III. G. L., a fifty-one year old Negro, was admitted to the Philadelphia General Hospital on February 28, 1952. He had been "weak and sickly" all his life but had no serious illnesses until January 30, 1952, when he was admitted to a hospital in an unconscious state. He was discharged on February 19, 1952, with a diagnosis of glomerulonephritis. On the day before admission to the Philadelphia General Hospital he remained in bed because of weakness. About six hours after admission he developed a severe headache and shortly thereafter became semi-comatose. There was no history of alcoholism.

He was lethargic, confused and barely coherent. His temperature was 98.6°F., pulse rate 80 per minute and blood pressure 220/110 mm. Hg. There was grade 2 hypertensive retinopathy. The apex beat was in the fifth interspace, 11.5 cm. to the left of the mid-sternal line. There was a grade 2 apical systolic murmur. His liver was palpable one fingerbreadth below the costal margin in the right mid-clavicular line. There was weakness of the left leg and arm, and with the exception of the right upper abdominal reflex, the cremasteric and abdominal reflexes could not be elicited. The remainder of the examination was normal. His spinal fluid was clear and had a pressure of 220 mm. water.

The weakness of the left arm and leg cleared in twenty-four hours and the confusion in approximately one week. Two days after admission considerable itching of his skin developed. At this time the sclerae were noted to be icteric.

His hemoglobin was 8.1 gm., white blood cell count 8,600 per cu. mm., reticulocytes 5.1 per cent and there was positive sickling on the peripheral blood smear. There was three plus albuminuria, 5 to 7 leukocytes and 3 to 5

TABLE IV

CASE II—LABORATORY DATA*

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism‡							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct†	Total	Bile	Urobilinogen	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu. mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg.	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr. (up to 1/20)	500–800 mg. %	(9–10 mg. % P)	(7.2–16.2 mEq./L.)	150–230 mg. % (150–250 mg. %)	50–30% of total (up to 30% of total)	50–70% of total (70% of total)	Up to .5 (up to .3)	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose—5 mg./kg. body wt. Normal less than 5% in 45 min.
3/2/48	8.0	2.9	....	8.3	.....	...	...	.....	...	.....	.....	272	128	144	.44	.....	0	0	1.0	0	0	0	7.3	15.3
3/4/48	8.5	3.1	2.6	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....
3/5/48	....	...	....	....	.....	...	0	(1/300)	...	.....	.....	218	100	118	.46	.....	...	...	...	..	..	...	.....	....
3/6/48	....	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	0	0	1.0	0	0	0	16.7	17.5
3/16/48	7.5	2.8	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	4.32	....
3/22/48	....	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	7.36 3.59/3.77	...	...	...	..	..	...	.....	....
3/29/48	....	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	7.04 3.12/3.92	...	...	...	..	..	...	.....	....
4/5/48	....	...	....	....	.....	...	...	(1/30)	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....
4/6/48	....	...	....	....	Immed.	1.5	...	.....	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....
4/8/48	....	...	....	....	Immed.	3.0	...	.....	...	.....	.....	....	...	...	...	6.96 3.85/3.11	...	...	...	..	..	...	.....	....
2/28/50	6.5	...	8.6	6.8	2.8	5.1	...	(1/40)	...	.....	.....	....	...	...	...	6.00 2.99/3.01	...	...	...	..	..	...	.....	19.8
3/2/50	....	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	0	0	1.0	0	0	1+	.....	....
3/6/50	10.5	...	....	5.9	del.	2.0	...	.....	...	.....	.....	258	66	192	.26	6.54 3.65/2.89	...	...	...	..	..	...	.....	16.4
3/7/50	....	...	....	....	2.4	3.5	...	(1/20)	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....

TABLE IV (*Continued*)

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism‡								Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct†	Total	Bile	Urobilinogen	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol					Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu. mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg.	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr. (up to 1/20)	500–800 mg. %	(9–10 mg. % P)	(7.2–16.2 mEq./L.)	150–230 mg. % (150–250 mg. %)	50–30% of total (up to 30% of total)	50–70% of total (70% of total)	Up to .5 (up to .3)	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose—5 mg./kg body wt. Normal less than 5% in 45 min.
3/8/50	....	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	2.2	....
3/9/50	11.0	...	....	....	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	2+	2+	1.0	0	0	0	.....	....
5/17/50	9.5	3.6	3.6	10.8	Immed.	2.0	...	.....	...	.....	.....	....	...	...	...	5.84 2.86/2.98	...	...	...	..	..	...	6.0	....
5/19/50	11.0	3.8	....	5.5	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	0	0	1.0	0	0	0	.....	....
7/26/50	5.0	2.5	8.0	6.2	.....	...	...	.....	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....
4/16/52	7.9	2.3	13.2	9.4	2.4	4.3	...	.....	...	.....	.....	....	...	...	...	6.08 3.25/2.83	0	0	1.0	0	0	0	25.6	30.0
2/3/53	6.6	2.7	....	6.5	.....	...	1+	5.6	...	.....	.....	....	...	...	...	.....	...	...	...	..	..	...	.....	....
2/4/53	7.8	2.6	5.3	....	.....	...	1+	.....	692	.....	.....	250	116	134	.47	5.6 3.05/2.55	...	...	...	..	..	...	.....	....
2/5/53	....	...	....	....	2.3	3.8	0	(1/20)	840	(23.9)	(15.3)	(325)	(87)	(238)	(.27)	5.84 3.52/2.32	0	0	1.0	0	0	0	29.0	....
2/7/53	9.8	3.3	....	....	2.3	4.3	±	.....	...	.....	.....	....	...	...	...	.....	0	0	1.0	0	0	0	23.6	....

* Abbreviations: R.B.C.—red blood cells; Retic.—reticulocytes; W.B.C.—white blood cells; CCF—cephalin cholesterol flocculation; TT—thymol turbidity; TF—thymol flocculation; SCG—scarlet colloidal gold; SR—scarlet red.

† In some instances the direct reacting serum bilirubin was determined qualitatively, del.—delayed; immed.—immediate; neg.—negative.

‡ Determinations indicated by parentheses in tests "Related To Lipid Metabolism" were performed in the laboratory of Dr. I. J. Pincus, Jefferson Medical College.

hyaline casts per high power field. The concentration test revealed a fixed specific gravity of 1.010. Phenolsulfonthalein excretion was 5 per cent in fifteen minutes. The blood urea nitrogen was 31 mg. per cent and the Wassermann test was negative. X-ray studies showed a prominent left ventricle. He was discharged on March 21, 1952, with a diagnosis of hypertensive encephalopathy, chronic pyelonephritis, anemia due to toxic suppression of the bone marrow and a sickling tendency.

In June, 1952, he was readmitted because of jaundice and pruritus. For the preceding two months he had experienced episodes of severe, colicky, right upper quadrant pain which, during the past three weeks, had been referred to the right subscapular region. No alleviating or aggravating factors were observed. There was an intolerance of fried and fatty foods. For the previous two weeks there had been pruritus, light brown stools and dark urine, and anorexia for one week. He gave a history of angina of effort, and for the preceding two weeks had noticed swelling of his feet and abdomen.

The physical findings were the same as those on the earlier examination except that his blood pressure was 145/70 mm. Hg and his neck veins were distended. The liver was palpable two to three fingerbreadths below the costal margin in the right mid-clavicular line. Its edge was firm and smooth. There was moderate ankle edema. The stools were light brown. His hemoglobin was 5.6 gm. and there were sickled and nucleated erythrocytes on the peripheral blood smear. A diagnosis of sickle cell anemia was made. Renal function studies were similar to those on the previous admission. The electrocardiogram was compatible with a diagnosis of left ventricular strain secondary to hypertension. A flat film of the abdomen revealed numerous calcified stones in the gallbladder region. During this admission there were several episodes of diffuse abdominal pain which were each of approximately twenty minutes' duration, unaccompanied by other symptoms and relieved by oxygen. He was given three blood transfusions and discharged September 3, 1952. Some of the more relevant laboratory findings during this and subsequent admissions and outpatient visits are recorded in Table v.

Several weeks after discharge, he felt progressively weaker, had frequent nose bleeds, and there was an occasional recurrence of the abdominal pains previously experienced. Ankle edema persisted. During the next few months there was persistent icterus with total serum bilirubin values ranging from 3.71 to 5.97 mg. per cent. The hemoglobin varied from 6.3 to 7.9 gm.

On December 1, 1952, he was readmitted because of dyspnea on slight effort, frontal headaches and ocular pain with blurring of vision. His appetite and dietary intake had been poor for one month and he had vomited two or three times weekly during the preceding three weeks. The vomitus did not contain blood. His stools were black but contained no frank blood. He was listless and lethargic but in no acute distress. He was 5 feet 4 inches tall and weighed 107 pounds. His sclerae were icteric. There was some distention of the neck veins. The tender liver edge was palpable two fingerbreadths below his costal margin in the right mid-clavicular line. The remainder of the physical findings were the same as on previous examinations. Radiologically, his stomach and duodenum were normal. The gallbladder could not be visualized by cholecystography but there were numerous, faceted, calcific stones in the gallbladder area. In view of the history and x-ray findings he was considered for cholecystectomy but rejected because of the serious operative risk. It was difficult to be certain whether hepatocellular disease or choledocholithiasis was present in addition to the hemolytic jaundice.

On February 23rd, the liver was three fingerbreadths below the costal margin in the mid-clavicular line and four fingerbreadths below the xiphoid process. Its edge was sharp, round, smooth and moderately tender. The spleen was not palpable. There was no jugular venous distention. A liver biopsy was performed at 8:55 A.M. after he had been fasting for twenty hours. Venous blood was withdrawn for tests between 8:44 and 8:58 A.M. A very small specimen of liver tissue was obtained which did not permit adequate histologic interpretation. Paper electrophoretic studies of serum proteins revealed no abnormalities. The results of electrophoresis by the Tiselius method are recorded in Table II. The amount of albumin was somewhat reduced. The new component noted in the serum of the patient in Case II was also present but in smaller amount. There might also be a small amount of fibrinogen present. The serum amylase level was 211 mg. per cent and serum lipase 1.1 ml. N/20 NaOH.

On March 5, 1953, he experienced an attack

of severe, sharp bilateral abdominal pain below his umbilicus. Peristalsis was hyperactive. There was no rigidity or guarding. For the following two weeks he had clay-colored stools. On March 25, 1953, the findings on examination of his liver were the same as at the time of the first biopsy. A second biopsy was performed at 8:45 A.M. after he had been fasting for approximately fifteen hours. Again, only a small specimen was obtained. Blood for laboratory tests, the results of which are recorded in Table V, was taken between 8:43 and 8:53 A.M. The blood sugar concentration was 81 mg. per cent.

The striking change on microscopic examination of the specimens obtained at both biopsies was the presence of abnormal amounts of fibrous tissue. (Fig. 7.) There was not enough tissue to determine the type of cirrhosis with certainty but the distribution of the connective tissue favored portal cirrhosis. In the parenchymal and Kupffer cells there were large deposits of pigment which gave a positive reaction for iron. Hemosiderin was also present in the fibrous tissue. There was only a little parenchymal cell degeneration and the involved cells were adjacent to the connective tissue. No apparent, striking pathogenetic relationship between degenerating iron-laden parenchymal cells and the formation of fibrous tissue was present as in Case IV. The sinusoids were widely dilated. In some areas they were filled with clumped red blood cell masses. A large area of parenchymal cell necrosis was found adjacent to an area where the engorgement of sinusoids by clumps of red blood cells was marked. (Fig. 8.) Kupffer cells were only slightly prominent.

Histochemical observations performed on the tissue obtained at the first biopsy are recorded in Table III. Because of the meager amount of tissue upon which these are based they are of little real value. The cytoplasm of the few cells which could be studied contained moderate amounts of glycogen. No nuclear vacuolation was noted. Nuclear desoxypentose nucleic acids but not cytoplasmic pentose nucleic acids may have been reduced in amount.

CASE IV. E. G., a twenty-five year old Negress, was first admitted to the Graduate Hospital, April 12, 1949. During the preceding evening she began to experience a dull, constant throbbing, pain in the left upper quadrant of the abdomen. This increased in severity, spread over the entire abdomen, then gradually subsided on the morning of admission. Residual discomfort remained in the right upper quadrant for a few days, accentuated by motion. Enquiry and subsequent reports from other hospitals revealed that at the age of two years a moderately severe anemia and sickling of her red blood cells had been discovered. She received blood transfusions for anemia at the age of eleven years. Splenomegaly was discovered at this time. When she was twelve years old she was observed in a crisis of sickle cell anemia with generalized pains, fever to 103°F. and marked anemia. From the age of twelve to nineteen years she had twenty hospital admissions, mostly for blood transfusions. Hematologic studies at the age of nineteen years showed that the hemoglobin was 5.4 gm., red cell count 1,330,000 per cu. mm., white cell count 15,000 per cu. mm. and the presence of marked red blood cell sickling.

Intermittent throbbing pains of short duration in varying parts of her body as well as episodes of scleral icterus had been recurring for as long as she could remember. Climbing one flight of stairs resulted in weakness and some dyspnea. Ankle edema had been present nightly for about five years.

Her past history included an illness diagnosed as rheumatic fever at the age of five years with swollen, tender joints of the fingers, wrists and knees. One brother was receiving care for sickle cell anemia in this hospital.

She was well developed and well nourished, alert, cooperative and in only mild discomfort. Her temperature was 101°F., pulse rate 102 per minute, blood pressure 120/70 mm. Hg. The apex beat was palpable in the anterior axillary line in the fifth left interspace. There was a grade 3 shrill, blowing systolic murmur at the aortic area and a grade 1 systolic murmur at the apex. Generalized guarding and tenderness of the abdomen, especially in the right lower quadrant, was present. Rebound tenderness was moderate and generalized and peristalsis was hypoactive. Apart from venous congestion of the retinal vessels, there were no other findings on examination. The hemoglobin was 6.0 gm., red blood cell count 2,040,000 per cu. mm., and reticulocytes 15.1 per cent. The blood smear revealed marked erythrocyte sickling, polychromatophilia, nucleated red blood cells and Howell-Jolly bodies. A diagnosis of sickle cell anemia in crisis was made. During the next two days her symptoms and fever subsided. A plain film of the abdomen showed evidence of slight hepatomegaly. An x-ray of the chest revealed

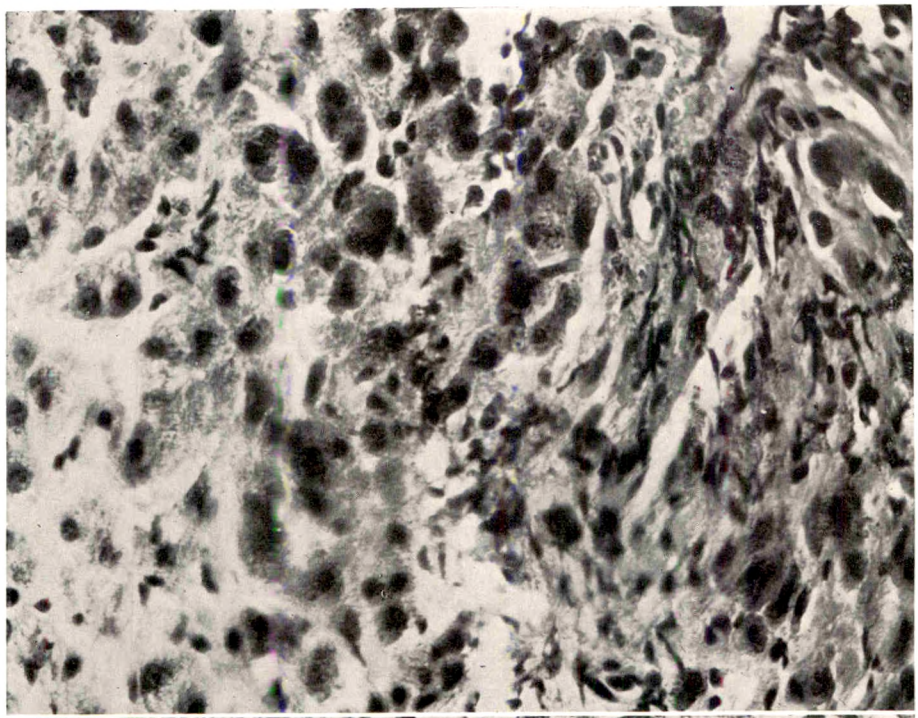
TABLE V

CASE III—LABORATORY DATA*

Date	Hemoglobin	Hematocrit	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism**							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention (%)
					Direct	Total	Bile	Urobilinogen	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	14.5–15.3 gm./100 cc.	47 cc./100 cc.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg. %†	Up to 1.0 mg. %	0	0–4.0 E. units /24 hr.	500–800 mg. %†	(9–10 mg. % P)	(7.2–16.2 mEq./L.)	110–300 mg. % (150–250 mg. %)	20–40% of total (up to 30% of total)	60–80% of total (70% of total)	Up to .4 (up to .3)	6.3–8.0 gm. % 4.0–5.5/1.3–3.4 gm. % 6.0–8.0 gm. %† 4.5–5.0/1.5–3.0 gm. %	0–1+†	2+ 2+†	1.0–4.0 1.0–4.0†	0–2+ 0–2+†	0–2+†	0–2+†	1.5–4.5 Shin. units 1.5–5.0 Bod. units†	Dose—5 mg./kg. body wt. Normal less than 5% in 45 min.
2/29/52	8.1	..	...	8.6	....	.....	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
3/3/52	...	..	...	...	....	.85	..	.....	....	.....	.....	.....	....	....	....	.....	....	0	2.5	0	..	..	....	....
3/7/52	9.6	28	5.1	...	....	.....	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
6/12/52	5.6	..	...	9.0	....	10.6	0	.....	....	.....	.....	167	102	65	.60	7.0 3.4/3.6	....	4+	5.7	3+	..	..	5.45	....
6/19/52	...	..	...	...	....	2.8	..	6.53	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
7/5/52	...	..	...	...	....	3.1	..	4.29	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
8/21/52	...	..	...	...	....	3.4	..	.....	....	.....	.....	.....	....	....	....	.....	....	2+	1.5	1+	..	..	....	....
12/1/52	4.7	..	...	...	....	.....	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
12/4/52	7.4	..	...	...	....	5.3	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
12/15/52	6.0	..	...	...	....	2.8	..	.....	....	.....	.....	.....	....	....	....	.....	....	0	1.6	1+	..	..	....	....
12/17/52	...	..	...	...	....	.....	0	0	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	....
12/18/52	...	..	...	...	....	.....	..	1.24	....	.....	.....	109	53	56	.49	.....	....	0	1.2	0	..	..	7.57	....
1/7/53	...	..	...	...	....	1.3	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	1.87	....
1/12/53	...	..	...	...	....	.....	..	.....	....	.....	.....	.....	....	....	....	.....	....	1+	7.4	3+	..	..	4.26	....
1/19/53	...	..	...	...	....	.....	..	.....	....	.....	.....	.....	....	....	....	6.5 3.7/2.8	....	4+	2.5	0	..	..	....	....
1/21/53	...	..	...	...	....	.....	..	.....	....	.....	.....	.....	....	....	....	.....	....	....	....	...	..	..	....	40

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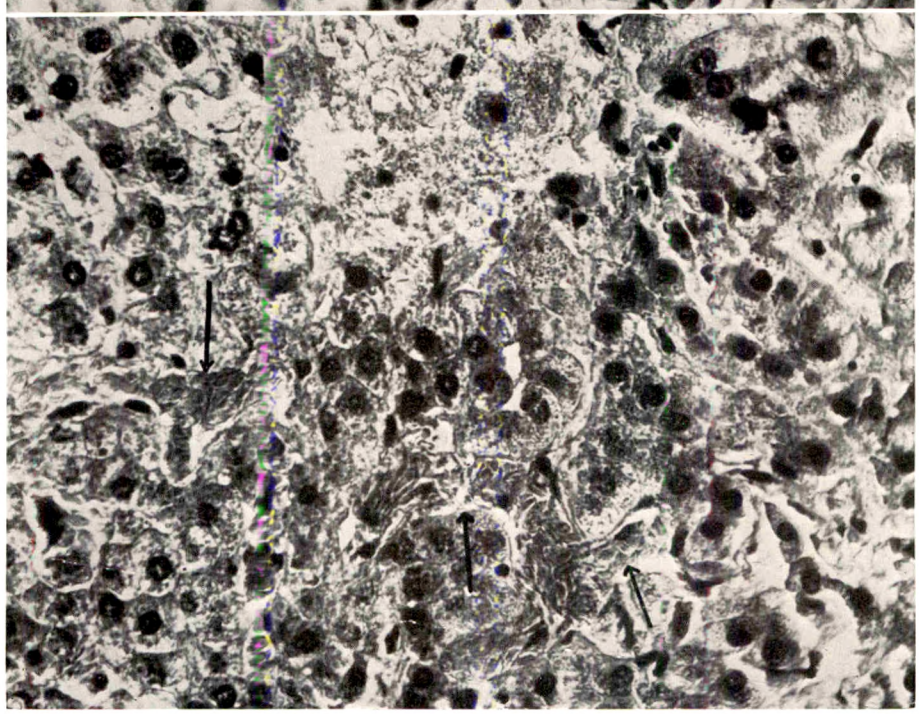


FIG. 7. Patient No. 3; high power view of fibrous tissue and adjacent degenerated parenchymal cells. In both the parenchymal cells and connective tissue are fine deposits of hemosiderin. Paraffin section (7 micra); hematoxylin and eosin; × 560.

FIG. 8. Patient No. 3. Adjacent to the area of parenchymal cell necrosis, there are many dilated sinusoids that are filled with clumped red blood cell masses (indicated by arrows). Paraffin section (7 micra); hematoxylin and eosin; B and G filters; × 350.

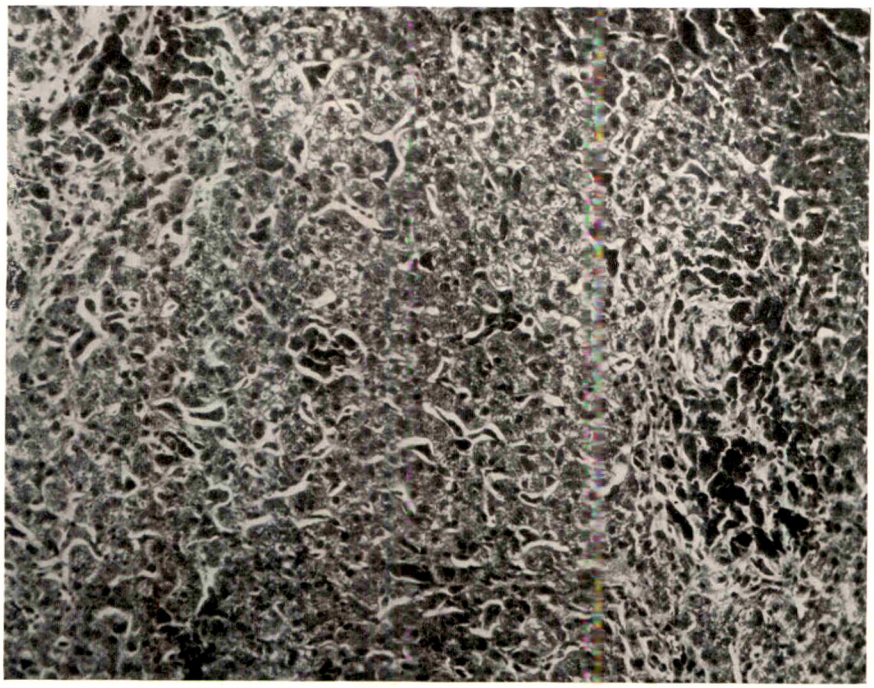


FIG. 9. Patient No. 4. Near the center of the photomicrograph is a focal area of degenerated parenchymal cells heavily laden with hemosiderin. In the upper left and lower right corners, fibrous replacement of the involved cells is prominent. Plugs of hemosiderin are seen in canaliculi, and Kupffer cells are filled with this pigment. Paraffin section (7 micra); hematoxylin and eosin; B and G filters; × 110.

in almost all of the parenchymal cells, in the Kupffer cells, and as plugs in the canaliculi. Focal areas of parenchymal cells containing iron were prominent. They showed no distinct zonal distribution. Frequently, bands of connective tissue, in which were deposits of iron, radiated from these sites to the portal areas. Deposits of hemosiderin were also prominent in the portal areas in which there was an increase in connective tissue. There were varying degrees of degeneration of parenchymal cells adjacent to connective tissue areas. Of interest were the deposits of iron in the arterial walls. There were only occasional small focal lesions in which parenchymal cells had "fallen out." Mallory stain revealed an increase in connective tissue of the portal areas and in areas in which iron-laden, degenerating parenchymal cells were replaced by fibrous tissue. The sinusoids were moderately dilated. No sinusoidal red cell clumps such as described in Case III were found.

As recorded in Table III, histochemical studies revealed no lipids in the biopsy specimen. The cytoplasm of the parenchymal cells contained a moderate amount of glycogen. About 1 per cent of the nuclei were vacuolated. There was probably no reduction in cytoplasmic pentose nucleic acids. Massive deposits of hemosiderin filled the parenchymal and Kupffer cells. No bile pigments could be seen.

COMMENTS

Liver Function Tests. Abnormal results for one or more of the "liver function tests," suggesting hepatic dysfunction, occurred in all of our patients. An increase in the serum concentration of direct-reacting bilirubin was almost always noted when hyperbilirubinemia was present and, in the absence of extrahepatic obstruction, was attributed to hepatic parenchymal involvement. A similarly high incidence of elevated direct reacting serum bilirubin levels has been reported by others. Thus of a total of fifty-one routine serum bilirubin determinations made by Henderson[2] in fifty-four patients with sickle cell anemia, twenty exhibited elevation of the direct-reacting bilirubin. Positive indirect reactions predominated during quiescent phases of the disease. Marked increases in direct-reacting bilirubin were associated with the largest and most painful livers although elevation of the indirect fraction also accompanied

TABLE VI

CASE IV—LABORATORY DATA*

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct†	Total	Bile	Urobilinogen‡	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu. mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg. %	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr. (Up to 1/20)	500–800 mg. %	9–10 mg. % P	7.2–16.2 mEq./L.	150–230 mg. %	50–30% of total	50–70% of total	Up to .5	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose—5 mg./kg. body wt. Normal less than 5% in 45 min.
4/12/49	6.0	2.0	15.1	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
4/13/49	10.0	3.6	13.9	8.5	.....	1.4	..	.....	...	..	..	220	100	120	.45	6.48 3.92/2.56	...	...	...	...	...	...	...	....
4/15/49	....	...	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	0	1+	2.0	±	2+	2+	...	....
4/27/49	7.5	2.9	.4	2.2	Neg.	.3	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
8/14/49	9.5	3.7	....	....	Immed.	2.0	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
8/15/49	11.5	4.1	12.0	9.3	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
8/16/49	13.0	3.2	....	4.2	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
8/18/49	13.0	4.4	....	5.9	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
9/15/49	8.5	3.1	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
10/4/49	7.0	2.6	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
10/6/49	10.5	3.7	....	....	Neg.	.6	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
1/11/50	6.0	2.1	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
1/12/50	9.5	2.8	....	....	Immed.	3.0	..	(0)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
1/13/50	8.5	2.5	9.9	....	Immed.	4.0	..	(0)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
1/14/50	8.0	2.3	5.3	....	Immed.	2.0	..	.....	...	..	..	...	...	...	...	.....	0	0	1.0	0	0	0	...	....
1/16/50	11.0	3.1	1.7	40.0	Immed.	3.0	..	(1/20)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....

TABLE VI (*Continued*)

Date	Hemoglobin	R.B.C.	Retic.	W.B.C.	Serum Bilirubin		Urine		Related to Lipid Metabolism							Related to Protein Metabolism							Serum Alk. Phosphatase	Bromsulfalein Retention %
					Direct†	Total	Bile	Urobilinogen‡	Total Serum Lipids	Serum Phospholipids	Serum Fatty Acids	Serum Cholesterol				Total Serum Proteins Albumin/Globulin	CCF		TT	TF	SCG	SR		
												Total	Free	Ester	Free/Total Ratio		(24 hr.)	(48 hr.)						
Normal	13.0–15.0 gm./100 cc.	4.2–5.4 million/cu. mm.	Up to 1.5%	5–10 thousand/cu. mm.	Up to .4 mg. %	Up to 1.0 mg. %	0	.1 to 1.2 E. units/2 hr. (Up to 1/20)	500–800 mg. %	9–10 mg. % P	7.2–16.2 mEq./L.	150–230 mg. %	50–30% of total	50–70% of total	Up to .5	6.0–8.0 gm. % 4.5–5.0/1.5–3.0 gm. %	0–1+	0–2+	1.0–4.0	0–2+	0–2+	0–2+	1.5–5.0 Bodansky units	Dose—5 mg./kg. body wt. Normal less than 5% in 45 min.
1/17/50	10.0	2.8	1.3	10.0	Del.	1.5	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
1/23/50	9.5	2.8	....	....	Neg.	.2	..	.....	...	..	..	...	...	...	...	6.68 3.98/2.70	0	0	1.5	0	0	0	...	....
10/3/50	5.0	2.1	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
4/12/51	6.3	2.5	3.1	12.3	.3	1.3	..	.....	...	..	..	180	64	116	.36	6.32 3.65/2.67	0	0	2.0	0	0	0	.8	....
4/13/51	....	...	....	....	.....	....	0	(1/20)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	36.0
4/16/51	11.0	3.8	1.3	....	.35	.88	0	(0)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
8/10/51	5.0	1.8	....	6.0	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
4/18/52	5.5	2.0	....	....	.....	....	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
10/4/52	9.3	3.3	....	....	.27	.75	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
10/10/52	....	...	....	....	0	.3	..	.2	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
12/19/52	10.3	3.6	....	....	Del.	.8	..	.....	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
3/31/52	7.0	2.8	....	....	.....	....	0	(0)	...	..	..	...	...	...	...	.....	...	...	...	...	...	...	...	....
4/1/53	8.0	2.4	....	5.7	.37	.81	..	.....	434	..	..	168	138	30	.82	7.52 4.18/3.34	2+	2+	3.5	2+	±	±	2.4	....

* Abbreviations: R.B.C.—red blood cells; Retic.—reticulocytes; W.B.C.—white blood cells; CCF—cephalin cholesterol flocculation; TT—thymol turbidity; TF—thymol flocculation; SCG—scarlet colloidal gold; SR—scarlet red.

† In some instances the direct reacting serum bilirubin was determined qualitatively: del.—delayed; immed.—immediate; neg.—negative.

‡ For values in parentheses all tests positive in dilutions less than 1/20 are recorded as 0.

hepatomegaly. Henderson suggested that when cholelithiasis is excluded the presence of direct-reacting bilirubin suggests associated "cholangiolar dysfunction." Green[8] and his coworkers observed that in some patients very marked elevation of serum bilirubin occurred on occasion, usually during crises. Their cases also showed a significantly high proportion of increased direct-reacting bilirubin levels, bilirubinuria and marked evidence of hepatic dysfunction.

Although Wintrobe[37] has stated that "remissions in this disease are never of such degree that no icterus was present," our patient in Case IV had normal direct-reacting and total serum bilirubin values on several occasions throughout the four-year period of observation in this hospital. Increased urinary urobilinogen excretion due to hemolysis, hepatic disease or both occurred at varying periods during the illness of all but the patient in Case IV. Bilirubinuria was demonstrated in Cases I and II. It is likely that had urinalyses been performed at appropriate times the remaining patients also would have exhibited bilirubin and excess urobilinogen excretion. In those with marked "cholangiolar damage" studied by Henderson[2] there was a fall in the fecal urobilinogen excretion, whereas, without evidence of liver damage, the fecal urobilinogen was high.

The almost constant finding of hypoalbuminemia in Cases I, II and IV, and the frequently decreased levels in Case III is significant and probably indicative, for the most part, of hepatic dysfunction. Serum globulin values varied. They were usually elevated in Case I but usually normal in the others. However, hyperglobulinemia was present in the fourth patient at the time of her liver biopsy and in the patient in Case III when his second biopsy was taken. In this small group of patients there appeared to be no relation between the occurrence of sickle cell crises and the presence of hyperglobulinemia. Because the method used for fractionating serum proteins may not give adequate separation of albumin and globulins, the results of the serum proteins studied by the Tiselius method performed in three of the patients are of interest. As noted in Table II, there was a decrease in the proportion of albumin and an increase in globulins. Among the globulins, the gamma fraction was the one most consistently elevated although some increase in the alpha$_2$ fraction was noted in one patient (Case I) and in the beta fraction in another (Case II), while there was a tendency for the alpha$_1$ globulin to be decreased in all three. These observations generally agree with those reported by Murphy and Shapiro[38] for one case and by Fenichel, Watson and Eirich[6] for fifteen cases of sickle cell anemia studied electrophoretically. These abnormalities of serum proteins are relatively common in various diseases, especially those with some accompanying impairment of liver function such as acute hepatitis or cirrhosis.[39–44] Similarly, the occurrence of a fraction with a mobility between the alpha$_2$ and beta globulins has been noted in other diseases. It is associated with serum lipids and may represent lipoprotein.[45] Fenichel, Watson and Eirich[6] believed that the changes in the serum proteins found in their cases were secondary to the general disease process, especially in the liver, caused by the sickle cell anemia.

Following the initial abnormal cephalin-cholesterol flocculation test (forty-eight hours) in Case I, all subsequent determinations were normal. The results of this test were normal in the second and fourth patients and varied throughout the course of the illness in Case III. The initial thymol flocculation test was abnormal in Case I and the thymol turbidity was subsequently only minimally elevated on one occasion. All other determinations of these and the serum colloidal gold and scarlet red tests were normal. There was no correlation between serum protein disturbances and abnormalities in these "turbidity and flocculation" tests performed on the same bloods taken at the times of biopsy. The importance of serial determinations in assessing liver function tests is apparent from a study of the findings in our first patient. It is difficult to appraise reports dealing with abnormalities of these tests unless there is some indication of the number performed and their relation to the illness.

Serum alkaline phosphatase levels fluctuated but were usually elevated in three of the four patients. There was no constant relation between the levels of this enzyme and the histologic hepatic abnormalities or other biochemical findings. Before ascribing elevations in serum alkaline phosphatase to liver disease, the possibility that bone lesions are present must be considered. Radiologic evidence of bony involvement was present in only one patient (Case II). Because osseous changes in patients with sickle cell anemia may not be radiologically

evident, it is still possible that these were present in the other two patients studied. Unless the possibility of bony involvement is considered, the use of serum alkaline phosphatase determinations in conjunction with turbidity and flocculation tests to establish a differential diagnosis between hepatic and posthepatic jaundice may result in incorrect interpretations. When elevations of serum alkaline phosphatase levels are due to liver disease, it is of interest to postulate that one mechanism for the raised values may be canalicular narrowing and obstruction consequent to sinusoidal widening which is so common a finding in this disease.

As may be noted in Tables I, IV, V and VI, some abnormality of serum lipids existed in all four patients. In view of the small number of patients and the complex problems associated with the interpretation of serum lipid levels in relation to primary liver dysfunction, no conclusions can be drawn.

Bromsulfalein retention was borderline in Case I but considerably increased in the other three patients. However, the elevation in the patient in Case II must be considered in relation to the hyperbilirubinemia extant when the bromsulfalein retention was determined. The frequent elevation of serum amylase and lipase levels in our patients is of interest, as occurring in a disease whose abdominal manifestations may be confused with acute primary pancreatitis.

Histologic Findings. The occurrence of "interstitial fibrosis"[46] and of cirrhosis has been described in patients with sickle cell anemia. Green, Conley and Berthrong[8] found advanced, nodular cirrhosis in four of twenty-one patients with sickle cell anemia at autopsy. Two of these were considered to be of the postnecrotic variety and two were not classified. There were trivial pigment deposits in two of the cirrhotic livers but one contained large amounts of iron-positive pigment in the parenchymal and Kupffer cells without pigmentation of the scar. Three of our patients had cirrhosis. Of these, one (Case IV) had hemochromatosis which was probably related to multiple blood transfusions. A case of exogenous hemochromatosis in a child with sickle cell anemia has been described by Frumin and Miller.[47] In the first liver biopsy specimen of the patient in Case II in whom portal cirrhosis developed over a period of three years, there were moderate amounts of iron-containing pigment in the parenchymal and Kupffer cells but none in the fibrous tissue scars. Although there was insufficient tissue to make a diagnosis of the type of cirrhosis with certainty in Case III, it was considered to be portal in type. The parenchymal cells were well filled with hemosiderin and small amounts of iron-containing pigment were also present in the connective tissue scars. Although, unlike the patient in Case IV, no striking relation between degeneration and necrosis of iron-laden parenchymal cells and fibrosis was evident, the possibility that the cirrhosis was related to hemosiderosis, the result of breakdown of exogenous and/or endogenous red blood cells, cannot be excluded with certainty. If in Case III hemosiderosis was not the initiating cause, it might have acted as an accelerating factor in the development of the cirrhosis. The possibility that hepatocellular damage may occur should be considered when multiple transfusions are used in an attempt to maintain higher levels of hemoglobin in this disease.

In Case II there was no history of alcoholism, deficient diet or other known factors that might have played a role in the development of cirrhosis. The dietary intake of the patient in Case III was considered inadequate and may possibly have played some role. As has been noted by Green et al.,[8] chronic anemia would not appear to be an important factor in the pathogenesis of cirrhosis. Nor did chronic congestive failure appear to be related to the cirrhosis in our cases. The absence of intratrabecular fatty cysts in Cases II and III makes it unlikely that a fatty liver antedated the cirrhosis.[48] It is not possible to exclude an element of viral hepatitis with consequent cirrhosis in our three patients. It is possible, however, that the portal cirrhosis in these patients may be unrelated to any known pathogenetic factor. Certainly this is true of many patients without sickle cell anemia.

Varying degrees of hepatic parenchymal degeneration and/or necrosis were found in our cases irrespective of other pathologic conditions present. Green et al.[8] have reported the occurrence of tiny focal or large lesions where liver cells had been lost, focal necrosis of the liver parenchyma, centrilobular necrosis, as well as diffuse atrophy most noticeable in central zones. Small focal hepatic necroses have also been noted by Diggs and Jones.[49] Scattered large foci of necrosis of the liver have been described in a patient with sickle cell anemia by Kimmelstiel.[50] No vascular occlusion was found and he concluded that the lesions were the result of acute

vascular spasm. The occurrence of severe widespread necrosis has also been reported by Green et al.[8] Our findings in liver biopsy material permit a more accurate interpretation because of the elimination of possible alterations due to terminal states or postmortem changes.

Focal hepatic parenchymal lesions were present in all of our cases. These varied in type, size and number. None showed a distinct zonal relation. Some were small and appeared as if a few liver cells "had fallen out." In some the parenchymal cells were degenerated and the nuclei were pyknotic. In some focal areas necrosis was evident. (Fig. 3.) Reticular stains usually revealed collapse of the supporting reticulin. Similar small focal parenchymal lesions have been also described in patients with portal cirrhosis, diabetes mellitus and in other liver diseases.[7]

In patients with sickle cell anemia, several possible causes for these focal lesions deserve consideration. It is considered too that the various focal lesions may have independent causes and may vary in significance. The role of iron-containing pigment as a cause of focal parenchymal degeneration has already been noted.

Engorgement of dilated hepatic sinusoids by red cell clumps was present in patients in Cases II, III and IV and in the second biopsy of the first patient. The most striking example of focal hepatic necrosis apparently related to ischemia occurred in our Case III. As demonstrated in Figure 8, the sinusoids surrounding much of this focal necrotic lesion were obstructed by masses of clumped red cells. It is conceivable that lesser degrees of parenchymal cell degeneration or necrosis might also result from sinusoidal blockades by masses of clumped red cells. It is probable that this cause of focal parenchymal necrosis occurs only in sickle cell anemia.

That Kupffer cells, engorged by phagocytosed erythrocytes, might also result in sinusoidal obstruction has been suggested by Green, Conley and Berthrong.[8] Kupffer cell prominence of varying degree was a constant finding in our patients and was most marked in Cases III and IV. Usually the cytoplasm of the Kupffer cells was filled with débris, some of which stained as hemosiderin. Their nuclei sometimes bordered the clumps of red cells, which appeared to have been phagocytosed. However, clumps of red cells in sinusoids were usually unassociated with Kupffer cells, and extreme prominence of these phagocytes sufficient to cause sinusoidal obstruction or sinusoidal widening as described by Green et al.[8] did not appear to be a feature of our cases.

It is conceivable that the marked and unexplained narrowing of small arteries in our patient in Case II might predispose to thrombosis especially in the presence of sickle cell disease. It is of interest to postulate that in this patient the cardiac enlargement unassociated with hypertension, the pulmonary lesions and the hepatic cirrhosis might all be related to vascular changes similar to those found in the liver. However, the excellent collateral circulation within the liver would make it seem unlikely that partial arrest of the circulation in only some of the vessels of this size would account for the parenchymal changes noted. As has been indicated, a more plausible explanation for some of the parenchymal changes found is the occurrence of vascular obstruction more distally, in the hepatic sinusoids, by masses of sickled red cells.

It is conceivable that if the focal lesions are large and particularly when necrosis is striking, as illustrated in Figure 8, collapse of the supporting reticulum followed by fibrosis may supervene. It is considered likely that the absence of any distinct zonal distribution and the recurrence of these lesions might favor the development of a multilobular cirrhosis. However, in the limited tissue provided by needle biopsy no definite relationship could be shown between any of the focal lesions and the development of cirrhosis in these patients.

In addition to the possible vascular causes of the parenchymal lesions, it is interesting to postulate that during crises and/or periods of hemolysis, a "toxic agent," possibly liberated as the result of an antigen-antibody reaction, may act as a hepatotoxin and be responsible for some of the parenchymal changes described, such as occurred in our first patient.

Histochemical Observations. The histochemical findings in the liver biopsies of all four patients are essentially the same. (Table III.) In the cytoplasm of the parenchymal cells one noted mere traces of demonstrable lipids or none at all, moderate to large amounts of uniformly distributed glycogen and large amounts of pentose nucleic acids. In their nuclei, there may have been some decrease in the content of desoxypentose nucleic acids.

The results of the histochemical tests revealed

no significant or characteristic change in the liver in sickle cell anemia. Correlations could not be established between the histochemical observations and the various biochemical findings for this small group of patients.

In most textbooks of pathology severe anemia has long been stated to be a cause of fatty degeneration in various organs, including the liver. Moreover, the tendency to stasis and the obstruction of sinusoids by sickled cells is a further complication in this form of anemia. Even so, hepatic fatty metamorphosis was negligible in our cases as well as in those reported by Green, Conley and Berthrong.[8] This may be related to the chronicity of the disease and its onset in early life. The negligible amounts of histochemically demonstrable liver lipids is also of interest since the patients had been fasting for about twelve hours when the biopsies were taken. In view of previous observations,[7] this may be considered unusual and possibly reflect another effect of sickle cell anemia on hepatocellular function.

The individual differences in cytoplasmic glycogen are not remarkable and are such as might be anticipated for any group of subjects studied under the same conditions. The occurrence of glycogen within the parenchymal cell nuclei in two of the patients is abnormal but may be encountered in a variety of liver disorders.[7,51] Its cause remains unknown. Considering that the tissues were fixed in ethanol, usually for several days, the observations on nucleic acids are probably unreliable[51,53] even though consistent. The apparently large amounts of pentose nucleic acids in the cytoplasm, however, suggest ample cytoplasmic protein despite disturbances in plasma proteins suggestive of hepatocellular dysfunction.

SUMMARY

Clinical and biochemical studies and histologic and histochemical observations on liver tissue obtained by needle biopsy were made on four patients with sickle cell anemia. There was one instance of hepatitis, one of hemochromatosis and two of portal cirrhosis. Serial liver function tests were performed in each case. The significance of the tests in this disease is discussed and the importance of serial determinations is indicated. Because osseous involvement in the absence of radiologic evidence may occur in sickle cell anemia, the serum alkaline phosphatase test is of limited value as an aid in differentiating jaundice of hepatic from posthepatic origin. Hypoalbuminemia and hyperglobulinemia, with elevation of the gamma fraction and a decrease in the alpha$_1$ globulin values, were the most consistent serum protein alterations.

The finding of elevated serum amylase and lipase values in this disease is of interest in view of its abdominal manifestations which may be confused with acute primary pancreatitis.

Hepatitis in one patient was thought to be a manifestation of the sickle cell disease *per se*. Focal parenchymal lesions were found in the liver of this patient during the recovery stage and in the liver specimens of the other three patients. They varied in type and in size. The possible causes of these changes are discussed. Ischemia, the result of sinusoidal obstruction by red cell clumps, would appear to be the most likely cause of some of them. That some of the parenchymal changes may be due to a "hepatotoxin" associated with sickle cell crises deserves consideration.

The likelihood that some of the necrotic lesions, when prominent and repeated, may be followed by cirrhosis as another hepatic manifestation of sickle cell anemia is noted. The precise role of iron-containing pigment in the pathogenesis of the cirrhosis in one of our patients is in doubt. Hemochromatosis in one patient was unquestionably related to multiple blood transfusions. The possibility of hepatic damage consequent to multiple blood transfusions given to maintain hemoglobin levels in this disease deserves consideration.

Histochemical studies revealed no or only mere traces of demonstrable lipids in the cytoplasm of the parenchymal cells. This is of interest in view of the anemia extant in these patients who were fasting at the time of biopsy. No characteristic changes in glycogen or nucleic acids were found.

Acknowledgments: The authors gratefully acknowledge the advice given by Prof. C. H. Best. Dr. L. H. Beizer performed the hematologic studies, and Dr. I. J. Pincus some of the serum lipid determinations. Thanks are due to Dr. A. S. Frobese for surgical assistance. Dr. A. Kaplan took the liver biopsies of three patients and Dr. C. H. Stone, III, the biopsy of one patient. Dr. C. M. Thompson, Philadelphia General Hospital, kindly permitted the study of one of his patients. Drs. D. W. Clark and G. R. Williams performed the Tiselius and paper electrophoretic

studies respectively. Dr. H. E. Taylor, Vancouver, kindly prepared many of the photographs. Mr. O. T. George assisted with the histochemical studies which were supported in part by a grant from the National Research Council, Canada. Mrs. J. Krautheim and Mrs. J. S. Salter helped with the preparation of the manuscript.

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Recent Developments in the Field of Glycogen Metabolism and the Diseases of Glycogen Storage*

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In 1929 von Gierke described a disease characterized by enlargement of the liver and kidneys with extensive deposition of glycogen in these organs.[1] Following the original description of glycogen storage disease, some fifty additional cases reached the literature by 1946,[2] and many more have been diagnosed and reported since.[3] Although the disease is relatively rare, the nature of the derangement in carbohydrate metabolism has assumed considerable interest for both the biochemist and clinician.

Schoenheimer[4] showed that the glycogen isolated from von Gierke's original case could be degraded by minced normal human liver, demonstrating the likelihood that the diseased liver is deficient in a fundamental mechanism required for glycogen degradation. However, systematic approach to the study of the disorder was not possible until relatively recent years since the accumulation of biochemical knowledge concerning the enzymatic processes involved in the synthesis and degradation of glycogen in the body was necessary. Particularly outstanding contributions to this field have been made by Carl and Gerty Cori but other workers have shared in the development of this fundamental knowledge.[5]

Central to the entire system of carbohydrate metabolism are the processes of glycogenesis and glycogenolysis, that is, the formation and breakdown of glycogen. These reactions, in as much as they influence the availability of glycogen, affect the overall metabolism of the organism in a profound way such as (1) maintaining the blood sugar level for cerebral and other vital organ functions, (2) making available sources of energy for other synthetic processes (protein and fat) and (3) providing a store of reserve energy which may be made available in times of stress (starvation). Therefore, disorders of metabolism which limit the availability of glycogen exert far-reaching effects demonstrable chiefly as hypoglycemia and impaired protein synthesis and growth.

It is the purpose of this paper to consider the metabolism of glycogen in the normal individual in the light of newly developed biochemical concepts. With this as background, attention will be directed to the diseases of glycogen storage, and an attempt will be made to correlate the clinical, biochemical and pathologic observations with a view toward a more complete understanding of the nature of the defects in these disorders.

GLYCOGEN METABOLISM IN THE NORMAL SUBJECT

Molecular Structure and Distribution of Glycogen. Glycogen or animal starch is a branched polysaccharide composed entirely of glucose units.[6] These units are arranged in chains with linkage between the first and fourth carbon atoms of adjacent units. Branch points of the molecule are established with linkage between the first and sixth carbon atoms. The molecule has been compared to a tree with a single free glucose unit (reducing end) and successive tiers of non-reducing units in the 1–4 linkage. The tiers are the result of successive and increasing numbers of branch points. (Fig. 1.) This

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concept of structure has been presented by G. T. Cori[7] and its formulation is the result of a series of ingenious studies concerning the enzymatic mechanisms of synthesis and stepwise degradation of glycogen. Molecular weight of the branched molecule is polydisperse and is of the order of one to four million or greater.[8]

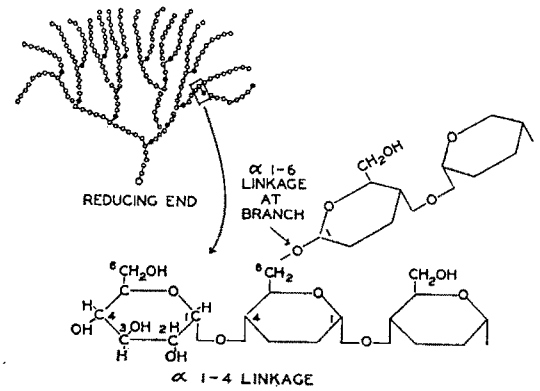
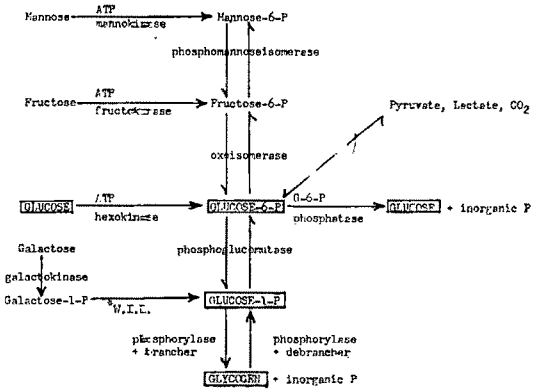


FIG. 1. The glycogen molecule as depicted by G. T. Cori.[18] Open circles are alpha 1–4 glucosidic links and solid circles are 1–6 links. Segment at the branch point is enlarged to show structure.

Glycogen formation occurs in practically every tissue of the body. Liver and muscle, however, are major sites of metabolism. Although small quantities may be found in most tissues, it is only in the liver and muscle that significant quantities are found. It is difficult if not impossible to state the normal range of concentration for liver and muscle glycogen since autopsy and biopsy specimens are subject to rapid glycogen breakdown. The nutritional status of the organism influences the level markedly and the degree of sympathetic nervous activity (epinephrine) alters the level. The capacity of the liver to store glycogen is not unlimited, and in rabbits fed high carbohydrate diets a glycogen level greater than 18 to 20 per cent is not exceeded, although under these conditions fat accumulates.[9] It is noteworthy, however, that in her studies of human glycogen Cori found concentrations ranging from 0.56 to 4.6 per cent in normal infant liver and 0.56 to 0.96 per cent in muscle specimens.[10]

Synthesis and Degradation of Glycogen. Ingestion of a variety of carbohydrates including glucose, fructose, galactose, and mannose lead to the deposition of glycogen in the liver. (Fig. 2.) Glucose is phosphorylated at the expense of ATP by the enzyme hexokinase, and the product glucose-6-phosphate is transformed by phosphoglucomutase to glucose-1-phosphate, which is the primary substrate from which glycogen is formed. Fructose is similarly phosphorylated by fructokinase and the fructose-6-phosphate derivative is converted by oxoisomerase to glucose-6-phosphate and hence to glucose-1-phosphate which gives rise to glycogen. Mannose is also



*Walden Inversion Enzyme.

FIG. 2. Enzymatic reactions involved in glycogen synthesis and degradation in liver.

phosphorylated to the 6-phosphate and in the presence of a phosphomannose-isomerase and oxoisomerase is converted to an equilibrium mixture of the 6-phosphate esters of fructose and glucose. Galactose is phosphorylated to galactose-1-phosphate by its specific kinase, after which a second enzyme system, Walden inversion enzyme, in the presence of a coenzyme uridine-di-phosphoglucose converts the product to glucose-1-phosphate.[11] Other metabolites are capable of forming glycogen if conversion to glucose-1-phosphate can be achieved. In this category are lactic acid, amino acids, propionic acid and glycerol. Following formation of glucose-1-phosphate, synthesis of the glycogen molecule succeeds by a progression of enzymatic reactions. Phosphorylase acts to remove the phosphate group from glucose-1-phosphate and attaches the bared first carbon atom to the fourth carbon of a glucose residue on the glycogen nidus.[12] This addition occurs on the non-reducing end of the molecule. In this way successively longer 1–4 glucose chains are added. This reaction is reversible and the direction, that is, glycogen synthesis as opposed to breakdown, is dependent on the ratio of inorganic phosphate to glucose-1-phosphate. At pH 7.0 synthesis occurs if more than one mole of glucose-1-phosphate per three moles of inorganic phosphate are in contact with the

enzyme. Thus a high level of inorganic phosphate favors glycogen breakdown. When the chain has been lengthened to a critical level of eight glucose residues, the molecule becomes the substrate for the second enzyme known as the branching enzyme, which transfers the alpha 1–4 linkage to an alpha 1–6 linkage, establishing a branch point of the molecule. Larner[13] very clearly demonstrated that this enzyme is indeed a transglucosidase by showing that on incubation with brancher glycogen containing C^{14} labeled 1–4 linkages developed 1–6 linkages which were labeled with C^{14}. In this way the glycogen molecule is synthesized.

Still another enzyme is required for degradation of glycogen since brancher activity is not reversible. This enzyme is called debrancher, has been isolated from muscle and liver[14] and causes hydrolytic cleavage of the 1–6 linkage, liberating glucose. It can only act, however, after phosphorylase, in the presence of inorganic phosphate, has systematically removed the 1–4 linked glucose residues from the outermost tier, converted these to glucose-1-phosphate and bared the branch points or the 1–6 links. Cori[15] has shown that phosphorylase degrades glycogen to a product called limit dextrin or LD wherein the outermost tier of glucose residues is removed to the branch points. Then debrancher acts and the penultimate tier becomes the outermost tier. Thus, by a series of orderly reactions the glycogen molecule is degraded. Because of the reversibility of the mutase enzyme the product, glucose-1-phosphate, is converted to glucose-6-phosphate. In liver and kidney this ester is split by a specific glucose-6-phosphatase to free glucose and inorganic phosphate, while in muscle, which lacks this phosphatase, the 6-ester is converted almost entirely to lactic acid or is oxidized. Free glucose liberated in the liver enters the blood stream, and in this way the importance of this phosphatase reaction for maintenance of a normal concentration of blood sugar may be seen.

Glycogen in the Fetus. Since certain of the disorders of glycogen metabolism are known to be familial and present at birth, it is of interest to consider the information available concerning the various glycogenic enzymes in the fetus. Studies of glycogen formation in the liver of the fetal guinea pig have demonstrated that three distinct phases are involved: (1) No glycogen is demonstrable in the liver before the fifty-seventh day of the sixty-six day gestation period. (2) Glycogen accumulates rapidly in the liver from the fifty-seventh day until term, when the concentration is two to three times that of the liver of the mother. (3) Liver glycogen is rapidly depleted following birth. In phase 1, all enzymes other than brancher enzymes concerned with glycogen synthesis are known to be present. It is thought that an increase in brancher initiates phase 2.[16] Nemeth has shown that glucose-6-phosphatase is absent until term.[17] Thus glycogen may accumulate in phase 2 since glucose-6-phosphate cannot be readily degraded and phosphohexokinase activity is relatively slow. Finally, concomitant with the appearance of glucose-6-phosphatase at birth glycogen is rapidly depleted. It is certainly of more than academic interest that a condition of glycogen storage, associated with a deficiency of the specific glucose-6-phosphatase, exists in the normal fetus.

Determination of Glycogen Structure. When the ability to synthesize glycogen is established in a tissue, molecular structure is dependent upon the ratio of activities of three enzymes, namely, phosphorylase, brancher, and debrancher.[18] Since the methods of analysis used by Illingworth and Cori[10] to determine the structure of normal glycogen have been utilized in the analysis of storage disease glycogen, these two methods are briefly outlined.

End-group analysis (enzymatic branch-point analysis): The glycogen sample is incubated with inorganic phosphate, phosphorylase and debrancher. Free glucose appears as a result of the debrancher action on each 1–6 link while glucose-1-phosphate appears as a result of the phosphorylase action on the 1–4 links in the outer chains. Thus, the ratio of 1–6/1–4 links is equivalent to the per cent of branch points and may be used in describing the configuration of the glycogen molecule.

Successive and alternating degradation using phosphorylase and debrancher: The first limit dextrin is isolated and by estimation of glucose-1-phosphate the outer chain length is determined. Debrancher action then determines the per cent of branch points. "After the first tier of branch points has been removed by action of glucosidase on LD_1, the debrancher enzyme is inactivated by heat and phosphorylase is added. This results in the formation of LD_2 which is again isolated, and so on. Each successive LD differs from the preceding one by the removal of one tier. Up to four successive LD's were isolated

from liver glycogen, by which time the degradation had reached 90 per cent. The analytical data obtained after each enzymatic step could be made to fit only one kind of model, namely, that which represents the polysaccharide as multibranched tree-like structures. If this structure were completely regular, there would be 50 per cent of all the branch points in the first tier, 25 per cent in the second, 12.5 per cent in the third, and so on. The percentages found in normal human liver glycogen were, however, 40, 25, and 10 per cent, respectively."[7] This implies a somewhat irregular structure.

Using these methods Illingworth, Larner and Cori[16] characterized the structure of normal rabbit glycogen in various states of nutrition, the structure of cat and fetal sheep glycogen and that of five samples of normal human glycogen.[10] Results are reproduced in Table 1 and indicate that human glycogen is very similar to animal glycogen in structure. Further, it is apparent that newly-formed glycogens after a period of fasting have longer outer chains and are less branched, while the reverse is true of older glycogens.

Factors Regulating the Concentration of Glycogen in the Tissues. One of the major defects in glycogen storage disease concerns the increased concentration of glycogen in the tissues. In considering the nature of the defect it seems worthwhile to comment on the variety of factors in the individual which influence tissue levels of glycogen. These factors are numerous and include hormones, electrolytes and enzymes; in addition, the nutritional state and certain diseases may affect these levels.

A well known phenomenon is the depletion of liver glycogen in fasted animals and the reverse situation in animals which are fed high carbohydrate diets. Stetten has shown in the rabbit that about 3 per cent of the ingested carbohydrate is converted to glycogen,[19] so that with an extremely high intake it is conceivable that liver glycogen may rise considerably. Further, any substance which increases the glucose uptake of tissues increases glycogen levels, and materials which inhibit glucose uptake have the opposite effect.

The role of insulin and the diabetogenic hormones must be mentioned in this regard. Insulin increases glucose uptake of isolated muscle[20] as well as glycogen synthesis in these tissues. An insulin effect upon hepatic glucose uptake has also been demonstrated but this is apparent *in vivo* only.[21] The exact mechanism by which this insulin action is accomplished is not clear, although an effect upon the hexokinase system is a favored hypothesis.[22,23] In experimental animals in the absence of insulin or in uncontrolled diabetes the liver glycogen is low.

TABLE 1
ANALYSIS OF GLYCOGEN STRUCTURE

Source of Glycogen	1–6 Links (%)	Degradation by Phosphorylase (%)
*Liver glycogen:		
Rabbit	6.8	36.2
Cat	8.1	28.0
Fetal sheep	8.7	36.6
†Human liver:		
Infant	7.1	31.9
Infant	8.4	41.0
Human muscle:		
Leg	8.0	34.6
Pectoralis	7.2	32.7
Pectoralis	8.1	25.0

* Data obtained from C. F. Cori.[7]
† Data obtained from B. Illingworth and G. T. Cori.[10]

Bondy[24] has also demonstrated that the human diabetic has decreased liver glycogen levels. A recent observation in the alloxan diabetic animal is of special interest. Langdon has shown that in diabetic rats the activity of the glucose-6-phosphatase of liver is markedly increased, and has suggested that this is in part responsible for an increased liberation of glucose from the liver in diabetes.[25] Administration of insulin results in fall in the activity of this enzyme to normal. In view of the fact that hyperinsulinism might conceivably lead to diminished glucose-6-phosphatase activity and increased glycogen storage, the author reviewed the autopsy findings in several patients who died of functional pancreatic adenomas. No evidence of increased glycogen in tissues could be found.[26]

With respect to inhibitors of glucose uptake, it is known that pituitary growth hormone and adrenal steroids decrease glucose uptake of muscle[27] and secondarily decrease glycogen synthesis. Adrouny and Russell[28] found that hypophysectomized rats rapidly deplete stores of liver glycogen and, on administration of

growth hormone, loss of glycogen from the liver is diminished. Further, in the normal fasted animal, with depletion of liver and muscle glycogen the levels of cardiac glycogen increase. This increase does not occur in hypophysectomized fasted rats unless a growth hormone is

TABLE II
FACTORS AFFECTING CONCENTRATION OF GLYCOGEN IN TISSUES

Increasing	Decreasing
High CHO intake	Starvation
Insulin	Insulin deficiency
Adrenal steroids*	Glucagon
Growth hormone*	Epinephrine
	Insulin antagonists (pituitary, adrenal)
	Diseases, i.e., thyrotoxicosis, cirrhosis of liver, uncontrolled diabetes

* See text for explanation.

administered. It is concluded, therefore, that the growth hormone is glycostatic with respect to the liver but actually increases the glycogen content of heart muscle.

Other substances known to affect glycogen levels include epinephrine and glucagon or alpha cell hormone of the pancreas. Sutherland[29] demonstrated *in vitro* that both of these substances, which *in vivo* raise the blood sugar and deplete liver and muscle glycogen, act upon phosphorylase and maintain the enzyme in its active form, phosphorylase-a. This increased glycogenolytic action, as opposed to the glycogenic action of phosphorylase, has not yet been explained. Further, although the role of epinephrine in the human subject is well established, that of glucagon is not. Some investigators believe that glucagon is a basic regulating hormone concerned with the maintenance of the blood sugar and McQuarrie has suggested that a glucagon deficiency exists in certain cases of hyperinsulinism.[30]

Hastings and his coworkers have been instrumental in pointing out the importance of electrolyte concentrations in processes of glycogen synthesis. He has shown that in order for the isolated liver tissue to synthesize glycogen, the tissue must be suspended in a medium containing high concentrations of potassium.[31] Muscle, on the other hand, will not synthesize glycogen in the presence of relatively great concentrations of potassium.[32] The significance of these observations in the intact animal is not yet clear, although it may be postulated that alterations in serum and tissue potassium, sodium and phosphate may well affect glycogen synthesis *in vivo*.

In general it may be said that the absence or decrease in activity of the glycogen-synthesizing enzymes could result in low glycogen levels while a decrease in the activity of the degrading enzymes could result in high concentrations in tissues. It is also conceivable that disproportionately increased activity of one group of enzymes as opposed to the others could influence the level of glycogen.

Those factors, then, which tend to accelerate the uptake of glucose by tissues produce increased levels of glycogen, while those depressing glucose uptake produce the reverse effect. (Table II.) Substances such as epinephrine and glucagon may conceivably influence glycogen levels by being present in greater or lesser quantities than normal. Finally, the activity of the enzymes concerned with glycogen metabolism may affect the glycogen levels. At the present time it is not known by what specific mechanism these factors regulate cellular glycogen levels but it is not farfetched to postulate that they do so by altering the enzymatic activity.

Before concluding this section it should be mentioned that certain disease processes appear to be associated with altered glycogen levels. Patients with thyrotoxicosis and cirrhosis of the liver tend to have low liver glycogen levels, the first presumably based on excessively rapid utilization, the second presumably associated with overall enzymatic deficiencies.[18] In diabetes, although liver glycogen may be low, one often sees increased deposits of glycogen in the kidneys and in the cardiac muscle[33] as well as in the beta cells of the pancreatic islets.[34] The basis for these deposits is not known although some investigators have suggested that hyperglycemia secondary to inadequate control of the disease may play a part. Finally it is of interest that the normal infant heart contains as much or more glycogen than many cases of cardiomegalic glycogen disease.[35]

Having considered the overall picture of the complexities of glycogen metabolism in the normal individual, the remainder of this paper will be concerned with the disorders of glycogen metabolism.

GLYCOGEN STORAGE DISEASE

Glycogen storage disease is a generic term applied to a group of congenital and familial disorders characterized by the deposition of abnormally large amounts of glycogen in the tissues. These diseases have been classified into four types on the basis of clinical, pathologic, and biochemical characteristics: Type I, von Gierke's disease or hepatorenal glycogenosis; Type II, glycogen storage disease of the heart; Type III, diffuse glycogenosis with cirrhosis of the liver; and Type IV, glycogen storage disease of liver and muscle.

This classification is based in part on that of Andersen[36] and in part on that of Cori.[18] For detailed and critical descriptions of these entities, the reader is referred to the papers of van Creveld,[37] Mason and Andersen,[38] Langewisch and Bigler,[39] di Sant'Agnese,[40,41] and Forbes,[42] as well as the standard textbooks of pediatrics.

Type I: von Gierke's Disease

Clinical Findings. This syndrome usually has its onset at birth or in early infancy. Often the only abnormality is the existence of an asymptomatic hepatomegaly. History of a sibling affected with this disorder may be the first clue to diagnosis. Symptoms may progress from anorexia, weight loss, abdominal enlargement, vomiting, and non-specific complaints to hypoglycemia, convulsions and coma. On physical examination there is found retardation of growth (occasionally dwarfism), obesity giving the child a "doll-like" appearance, hepatomegaly and palpable kidneys. Laboratory examination is marked by acetonuria, hypoglycemia, hyperlipemia, increased blood glycogen levels,[43] inadequate response to epinephrine, impaired glucose-tolerance, decreased serum phosphate and no response to glucagon.[44] The course is usually short but depends on the degree of involvement. Some patients live to adolescence. Death is usually due to intercurrent infection. Treatment consists in frequent high protein feedings and ACTH or cortisone[45] in an effort to control hypoglycemia. Sodium lactate is given for the acidosis which is often marked.[46]

Pathologic Findings. Andersen[36] has reported observations noted at autopsy on a patient with von Gierke's disease who lived for over two months, ultimately succumbing to a respiratory infection of two days' duration. The liver was massively enlarged, weighing 770 gm. (normal, 140 gm.), and the kidneys were moderately enlarged, with a combined weight of 58 gm. (normal, 40 gm.). Aside from pneumonia, no other abnormalities were observed upon gross examination. On microscopic examination, however, both the liver cells and the cells of the convoluted renal tubules were heavily loaded with glycogen; in addition, the liver cells contained an abundance of fat. Best's stain confirmed the large deposits of glycogen in the liver and kidneys and also revealed small amounts of glycogen in the heart, tongue and skeletal muscle.

Biochemical Findings. This disorder is characterized by hepatorenal deposits of metabolically unavailable glycogen. The unavailability of glycogen is attested to by the hypoglycemia and the absence of response to epinephrine. Ketosis and hyperlipemia are consistent with a shift to fat metabolism and reflect a carbohydrate-starved organism.

In an analysis of the defect several possibilities present themselves. Glycogen may be unavailable because of (1) a structural abnormality, which prevents the normal enzymes from degrading it, (2) a deficiency of the glycolytic enzymes or (3) an abnormality in the hormones or factors which regulate glycogen levels.

With regard to the structure of this glycogen it was known, following the experiments of Schoenheimer, that normal liver mince could degrade it, but it could not be degraded in the livers of patients with the disease. This suggested that its configuration was normal. In an analysis of the structure of the glycogen obtained from ten cases of this disease, G. T. Cori showed that the structure did not differ from that of normal. Phosphorylase degraded the molecule from 30 to 42 per cent while in the normal the values were 25 to 4[illegible] per cent. Further, the percentage of branch points was found to be 6.7 to 9.5 per cent while in the normal it was 7.1 to 8.4 per cent.[10]

In approaching the second possibility, that of enzymatic defect, the Coris reasoned that the glucose-6-phosphatase was most probably at fault. This was based on the fact that liver and kidney rather than muscle were involved by the disease and that the glucose-6-phosphatase is present in the former two organs and not in muscle. In seven cases the glucose-6-phosphatase activity of liver was demonstrated to be extremely low.[47] Activity of the enzyme in kidney

was also low in two cases. No evidence of significant inhibitors of this enzyme in these livers could be found. In five other older patients with a milder form of the disease, the phosphatase was only slightly depressed and in one case was within the normal range. Although this deficiency in glucose-6-phosphatase is an important factor in the mechanism of the glycogen storage, some question may remain as to whether it is the sole cause of this type of storage disease. It is possible that the low serum phosphate reported in these cases may also contribute to glycogen storage.

Type II: Glycogen Storage Disease of the Heart

Clinical Findings. The onset of this disease occurs early in infancy and is marked by feeding difficulties, intermittent cyanosis, and dyspnea. Cardiac failure, listlessness, muscular weakness, neurologic abnormalities and striking enlargement of the tongue may be noted. On physical examination, the diagnosis may be confused with hypothyroidism,[48,49] mongolism, or amyotonia congenita because of the extreme muscle hypotonia.[50] The heart is usually enlarged, and systolic murmurs are present in some cases. Laboratory studies reveal no abnormalities of carbohydrate metabolism. The cardiac silhouette on x-ray tends to be globular. Electrocardiogram reveals inverted T waves, left axis deviation and S-T depression with a normal P-R interval. The course is short with death resulting from infection and heart failure during the first eight months. No treatment is known.

Pathologic Findings. Pathologic findings in cardiomegalic glycogen disease have been summarized by di Sant'Agnese et al.,[41] who have reviewed fourteen cases previously reported in the literature. The outstanding findings revealed upon gross examination were in the heart, which in every case was strikingly enlarged, weighing in some instances 5 to 6 times the normal for the age of the patient.

Histologically, the marked infiltration of the myocardium with glycogen yields a distinctive microscopic picture, lending a "lacework" appearance to the fibers, each fiber appearing as a hollow cylinder surrounded by a thin, delicate cytoplasm with the nucleus lying free in the center. These alterations in structure are often so marked as to make recognition of the myocardial tissue difficult.

In six of the fourteen cases, glycogen was noted in the skeletal muscles. The tongue was consistently and markedly affected, the diaphragm less so, while the musculature of the neck, trunk and extremities contained variable amounts. Glycogen deposits were also observed in smooth muscle, the cells of the reticuloendothelial system, the kidney, the liver and in the reticulum cells of the spleen, lymph nodes, bone marrow and thymus.

Epithelial cells in which glycogen deposits have been noted are the mucus glands of the gastrointestinal tract, the acinar, ductal and islet cells of the pancreas, the cortical and medullary cells of the adrenals and, finally, the epithelial cells of the hair follicles. The ganglion cells and central nervous system may also be involved. There is no apparent reaction of the tissues to the accumulation of glycogen.[50]

Biochemical Findings. In this disorder of cardiac and generalized glycogenosis, no abnormalities of carbohydrate metabolism are found. This implies that the glycogen is available for utilization by the body. The Coris found that both the structure of the glycogen and the glucose-6-phosphatase activity of the liver and kidney were within normal limits in one carefully studied case. Since this disease affects chiefly muscle, one would not have anticipated a defect in the phosphatase system. At the present time no explanation of the abnormality is available.

Type III: Diffuse Glycogenosis with Hepatic Cirrhosis

Clinical Findings. Symptoms may appear at any time from late infancy to early childhood. The complaints are referable to hepatic involvement, with edema and abdominal swelling and occasionally bleeding tendencies. Upon physical examination hepatosplenomegaly, ascites, jaundice and anemia may be evident. The laboratory confirms abnormal liver functions (cephalin flocculation, thymol, bromsulfalein retention, low serum albumin and elevated serum globulins, bilirubinemia). The blood sugar is normal and no acidosis is present; response to epinephrine may be moderate and delayed. The glucose tolerance test may show a moderate rise and a slow fall of the blood sugar. The course is variable and death may occur between the ages of one to ten years.[36] Treatment is directed to the liver disease, although apparently it is not effective.

Pathologic Findings. Andersen has reported observations noted at autopsy of a seventeen month old child who died of glycogenosis with

hepatic cirrhosis; a previous sibling had died with hepatosplenomegaly and von Gierke's disease, the latter being diagnosed posthumously.

The liver weighed 560 gm. (normal, 330 gm.) and had a finely nodular surface. The spleen was also enlarged. Microscopic examination revealed a diffuse, finely nodular cirrhosis with large glycogen deposits in the liver cells; in the thickened, fibrotic portal areas were proliferating bile ducts, isolated liver cells and diffuse infiltration with round cells and polymorphs. In the spleen there were accumulations of what appeared to be reticulum cells loaded with glycogen; similar accumulations of phagocytic cells loaded with glycogen were found in the lymph nodes, the lymph follicles of the intestine and the intestinal mucosa; in all areas these phagocytic cells were accompanied by a mild fibrous reaction. Small amounts of glycogen were found in diaphragmatic muscle fibers but none were found in the kidneys.

Biochemical Findings. The pathologic findings in this variety of glycogen disease suggest that glycogen behaves as a foreign body to which there is tissue reaction, that is, cirrhosis of the liver and fibrosis surrounding the deposits of glycogen. This finding is totally consistent with the striking alteration in structure of this glycogen reported by Cori.[18] End group analysis revealed branch points of 4.7 per cent, phosphorylase degradation 50 per cent, average outer chain length 14.7 residues, and inner chain length 5.5. Thus there were fewer branch points and significantly longer chains. These findings suggest a deficiency of the brancher enzyme. The glycogen formed resembles amylopectin, the branched component of starch. Certain of the physical properties are consistent with this view and are markedly different from normal glycogens (x-ray diffraction diagram, solubility properties and absorption maxima after the development of the iodine color).

TABLE III
SUMMARY OF GLYCOGEN STORAGE DISEASES

Type	Organs Affected	Age		Hypoglycemia, Ketosis, Hyperlipemia	Epinephrine Response	Glycogen Structure	Enzymatic Alterations
		Onset	Death				
I	Liver; kidney	Neonatal; infancy	Varies with severity	++++	0-++	Normal	Decreased to absent glucose-6-phosphatase
II	Heart muscle, tongue, brain	Neonatal; infancy	Early	0	Normal	Normal	None found
III	Liver (cirrhosis); reticuloendothelial system	Late infancy	1-10 yr.	0	++	Abnormal: excessively long outer branches	Probably decreased brancher activity
IV	Liver; muscle	Late infancy	?	++	+	Abnormal: very short outer branches	Probably decreased debrancher activity

Type IV: Glycogen Storage of Liver and Muscle

Clinical Findings. This appears to be a very rare disorder. Only one case thus far recorded* was noted by Forbes.[42] In this case, there was no familial history of glycogen disease. Abdominal enlargement was first noted at the age of one year. Physical examination revealed only hepatomegaly. Mild hypoglycemia and acetonuria were noted. There was no bromsulfalein retention. A mild normochromic anemia was present. The patient was followed carefully and developed normally in every way. At twelve years of age, hepatomegaly was more striking and

* An additional case has been diagnosed by Dr. Alexis F. Hartmann at the Children's Hospital in St. Louis. Hypoglycemia, hyperlipemia and hepatomegaly were noted. The patient died at thirteen months of age with glycogen storage in the liver, heart and muscle. Glycogen structure showed findings similar to that of Forbes' case.

abdominal veins were prominent. Fasting ketonuria was noted. Cephalin flocculation was three plus, and marked bromsulfalein retention was found. The response to epinephrine was present but less than normal. It was of interest that over a ten-year period of observation the fasting blood sugar levels tended to rise and the lipids to fall. The patient, now thirteen years of age, is still living and does not appear chronically ill.

Pathologic Findings. Pathologic findings in this case have been limited to biopsy specimens taken at age twelve and a half. The liver specimens revealed parenchymal cells packed with glycogen, as identified with Best's stain; aside from this and some increase in periportal connective tissue, the liver was otherwise not remarkable. Routine hematoxylin and eosin sections of skeletal muscle revealed no abnormalities.

Biochemical Findings. High levels of glycogen were noted in muscle and liver biopsy specimens from this patient. The combination of glycogen storage in muscle and liver with a definite but abnormally small response to epinephrine suggests (1) that the glycogen is only partially available and (2) that the defect is probably unrelated to the glucose-6-phosphatase. Structural analysis of this glycogen was particularly interesting. Liver and muscle, respectively, showed end groups of 10 8 and 13.1 per cent and phosphorylase degradation of 12.2 and 2.6 per cent. These findings indicate an enormous number of branches and very short outer chains with the molecule essentially similar to limit dextrin. Cori suggests that there is a deficiency of debranching enzyme while phosphorylase is apparently normal. In this way the outer tier residues are readily released and rebuilt but the inner core is unavailable. This could explain glycogen storage and partial response to epinephrine.

A summary of the salient features of these four types of storage disease may be found in Table III.

SUMMARY

The field of carbohydrate chemistry and metabolism has advanced tremendously over the past twenty-five years. Elucidation of the enzymatic steps involved in the synthesis and degradation of glycogen has been one of the most important contributions. This has provided not only an understanding of glycogen metabolism in the normal individual, but has permitted an evaluation of glycogen storage diseases. Based on the clinical, biochemical, and pathologic data described, these diseases, once considered a single entity, have been classified into four types. Fundamental distinctions among these diseases have been demonstrated to reside in a variety of enzymatic defects in glycogen metabolism, resulting in increased tissue glycogen concentrations in all cases and in abnormal glycogen structure in some cases.

Acknowledgment: The valuable assistance of Dr. Robert E. Shank, Dr. Alexis F. Hartmann and Dr. Donald B. Rinsley is gratefully acknowledged.

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Conference on Therapy

Choice of Therapy in Intestinal Parasitic Disease

THESE are stenographic reports, which have been edited, of conferences by the members of the Departments of Pharmacology and of Medicine of Cornell University Medical College and New York Hospital, with collaboration of other departments and institutions. The questions and discussions involve participation by members of the staff of the college and hospital, students and visitors. A selected group of these conferences is published in an annual volume, Cornell Conferences on Therapy, by the Macmillan Company.

DR. GEORGE READER: The choice of therapy in intestinal parasitic diseases is the subject of this conference. The discussion will be opened by Dr. Kean.

DR. B. H. KEAN: We might modify the title a bit by saying that we will discuss only the more common intestinal parasitic diseases. There are a great many intestinal parasites but if we exclude about ten of the more exotic varieties, limiting ourselves only to those which we might encounter in the New York City area, we are left with about fifteen diseases. These include amebiasis, two flagellate diarrheas, five helminthic infections, four tapeworm diseases and schistosomiasis which is caused by a fluke. This is still an ambitious undertaking for one session and we shall try to deal only with as many as space will permit.

I should like to say at the outset that much that is written about the results of various treatments should be regarded with suspicion. It seems to be unusual to find an initial evaluation presenting the facts. For example, for many years we believed we knew how to treat ascariasis. We used to speak of cures in about 90 per cent of the cases and that is what one finds in almost any textbook. At the last meeting of the American Society of Tropical Medicine eight different persons from various parts of the world agreed that we are far from knowing how to treat Ascaris lumbricoides and that currently the cure rate is only about 35 per cent.

The situation in the case of pinworm is well known, for there is no ideal therapy for this disease. We shall discuss pinworm in more detail later.

Let us for a moment consider the most important of this group of diseases, that caused by Endamoeba histolytica or intestinal amebiasis. Even if we do not consider the archaic drugs which clutter up the field and mention only those currently in use, the list is large; emetine, various arsenicals like carbarsone,® milibis® and balarsen,® the halogenated hydroxyquinolines like diodoquin,® chiniofon, yatren® and vioform, a half dozen antibiotics, and some others which do not come to mind at this moment. Five or six years ago, when bacitracin became available, it appeared that the cure for amebiasis had been found. That illusion lasted only a year, for the cure rate dropped to about 20 per cent. Aureomycin was the next drug to appear, with an initial cure rate approximating 95 per cent. These rates have now dropped to about 30 to 50 per cent. The initial reports in the case of terramycin® were very optimistic. There was one report that sixty people were treated with terramycin, 2 gm. a day for about ten days, that the cure rate was 100 per cent, and that there were no toxic symptoms, diarrhea, nausea or complaints. It is my experience that you cannot give 2 gm. of terramycin a day to sixty patients without receiving complaints. Terramycin is still being used and is probably the best single drug for amebiasis but the ultimate evaluation of its efficiency requires an open mind. Fumagillin is another of the special cures for amebiasis. By the time the drug appeared on the market the cure rate was dropping from 90 per cent to 75 per cent; one can predict that in a year or so the cure rate will be still lower. It should be noted that terramycin affects bacteria to a much greater extent than does fumagillin. Tetracycline is being evaluated at the present time. Chlo-

roquine diphosphate might also be mentioned; it is virtually useless for intestinal amebiasis but very important for the treatment of hepatic amebiasis, in conjunction with emetine.

Emetine is, of course, used for both the intestinal and hepatic forms of amebiasis. There is something strange about this drug. It is the only one among all of the drugs previously mentioned that is a true amebicide, that is, it kills amebas in dilutions which may reasonably be expected to prevail with therapeutic doses. It is, of course, easy enough to set up test tube culture experiments and demonstrate amebicidal actions with terramycin or other drugs, but so far as we know none of these, with the sole exception of emetine, kills amebas in therapeutically relevant dilutions. Emetine is potent against the amebas in dilutions of 1:5,000,000 and it kills amebas in dilutions of 1:1,000,000. There is, of course, the difficulty that emetine is a fairly toxic substance. Strangely enough, in spite of its high amebicidal action, emetine alone never cures intestinal amebiasis. I do not have an adequate explanation for this phenomenon. Used in conjunction with certain other preparations, emetine is, of course, extremely effective in bringing about a cure.

Among the more popular members of the arsenical group, namely, carbarsone, balarsen and milibis, there is very little choice. We prefer carbarsone. There has been a great deal of experience with it over the years, and its toxicity is reasonably low for an arsenical compound. Some of you may remember the report by Anderson about two years ago in which he described the use of thioarsenites for amebiasis. This material has been found to be too toxic for clinical use and is not commercially available.

There is relatively little to choose from among the preparations of halogenated hydroxyquinolines. We prefer diodoquin. It seems to be the most popular among this group of materials. For many years it was the drug of choice in New Orleans where it cured about 95 per cent of cases of amebiasis. This hopeful report was followed by other reports from England which declared the drug to be almost worthless. The United States Navy refused to include diodoquin in its list of necessary drugs, mainly on the basis of the British experience. In recent years the experience in New Orleans seems also to show a falling off of the cure rate. At the present time it is probably closer to 50 per cent, although most of the reports still refer to from 60 to 80 per cent.

Since none of these drugs is entirely satisfactory, we pursue the plan of using several drugs, stretching the treatment over long periods of time. One plan we use in the treatment of amebiasis is to administer 1 gr. of emetine (65 mg.) intramuscularly daily for five days. It may be mentioned parenthetically that in the case of the five-day therapy electrocardiographic control is not necessary. It is desirable to use electrocardiographic checks when the treatment is carried on over a longer period of time, say ten days, as in the case of hepatic amebiasis or in older persons. With a shorter period of treatment some changes in the T waves may appear but they are unimportant from the practical standpoint; and if there has been no untoward reaction to the first dose of emetine, it is unlikely that anything serious will occur during the first five days. This is not true, however, with longer treatment. Simultaneously with the emetine we start carbarsone, 1 gm. daily, continued for ten days, one 0.25 gm. tablet being given four times daily. This is then followed by a course of terramycin, 1.5 gm. the first day and 1 gm. daily thereafter for a week. If the patient seems able to tolerate more terramycin, it may be given for ten days. This schedule constitutes our initial effort to cure a patient of amebiasis. We expect a cure with this method in about 90 per cent of the cases. If follow-up reveals that the parasite has returned, the whole course of treatment is repeated, adding a fifteen- or twenty-day course of diodoquin. We usually give 2.5 gm. daily in divided doses to an adult. This should result in the cure of a fair number of the 10 per cent failures. There is still a small residual group which is resistant to cure and which constitutes quite a problem.

DR. HARRY GOLD: Would your general formula of treatment apply to the asymptomatic form of amebiasis as well?

DR. KEAN: There has been some question and considerable debate about that over the years. Now, with the known danger of hepatic amebiasis, and with fuller recognition of the importance of the public health aspects of the disease, the debate is almost over. Consider for a moment what one means by a case of asymptomatic amebiasis. This is a patient who believes that he is perfectly well and may even regret that he has had the misfortune of having had his stool examined, as the result of which a parasite was found. The plan of therapy which I outlined might be accepted readily as appropriate

for acute amebic dysentery when it is necessary to alleviate the patient's symptoms. There may be a doubt, however, as to whether one is justified in using this heroic treatment in a person who seems perfectly well. Experience indicates that many people with so-called asymptomatic amebiasis who think they are well are not really in perfect health but are able to recognize this fact only when the so-called asymptomatic disease is cured. It is very much like a patient with low-grade anemia who does not know how well he can be until the anemia is cured. All patients with amebiasis should be treated. There is still some question as to whether the treatment should be as thorough in these cases as in the symptomatic ones. It is our inclination to treat them in the same way. We warn the patient that he may feel worse during the treatment but that he may anticipate feeling better after treatment than before.

DR. READER: We might ask if there are any questions about amebiasis before we go on to some of the other parasites. Dr. Almy, do you have any comment on the treatment of amebiasis?

DR. THOMAS P. ALMY: I was wondering about the feasibility of giving five daily injections of emetine to ambulatory patients who do not admit having any symptoms. Are you not forced at times to modify the schedule and, if so, just how do you do that?

DR. KEAN: You have called attention to a most important point, especially applicable to treatment in clinics of large numbers of patients. There we are inclined to eliminate emetine from the program and carry out only the other parts of the schedule. The pain of an injection of emetine is sometimes considerable. One cannot use a drug with the potential toxicity of emetine in hundreds of individuals in clinic populations without occasionally getting into some difficulty. We are less apt to encounter this trouble in the case of the more individualized care of private patients, and in such cases we are apt to carry through the full regimen of treatment. I would be interested in knowing what you think about this, Dr. Almy. Do you prefer to use one drug at a time and see how the patients get on, and, if they are not cured, then to turn to another drug or do you prefer to use several measures at the same time?

DR. ALMY: I have been using only one drug but am perfectly happy to use two or three. I would be very much interested in a convincing demonstration of the differences in the results.

DR. READER: Dr. LeMaistre, I wonder whether you would have any idea as to why terramycin is so effective and aureomycin is not?

DR. CHARLES A. LEMAISTRE: I am inclined to think that the difference between the two is accentuated by studies and data that are not of equal quality. On reviewing the data on aureomycin a few months ago I was impressed with the fact that the evaluation of this drug has not been as careful, extensive and thorough as it has been in the case of terramycin. It may well turn out that the two are not materially different, but at the present time, as Dr. Kean knows, I personally prefer terramycin.

DR. ALMY: I was rather cast down to hear Dr. Kean imply that an ancient explanation is probably not correct. It used to be said that emetine is ineffective against the cyst form of the ameba, and since the cyst form could not be eradicated from the lumen of the intestine it reinvaded the bowel and in that way prevented a cure. Is that now thought to be incorrect?

DR. KEAN: One cannot be certain whether it is or not. You all remember that amebiasis is usually acquired by drinking contaminated water. If the trophozoite is ingested in this way no harm is done because it does not survive the gastric barrier, even though this is the form of the ameba that is so dangerous to the host. On the other hand, ingestion of the cyst form gives rise to the disease because the cyst passes the stomach unharmed and in the intestine divides, yielding trophozoites. These trophozoites invade the mucosa, destroy portions of the submucosa, and lead to impairment of the blood supply with eventual ulceration of the mucous membrane. The question that has come up is this: Is the conversion from trophozoite to cyst a reversible process in the same patient? A certain number of trophozoites, instead of invading tissue, round up and form cysts which may be passed in the stool. Can these newly formed cysts divide and release trophozoites without crossing the gastric barrier of a new host? There is no final answer.

DR. READER: I wonder if Dr. Grace would care to express an opinion as to why some people develop acute dysentery from the ameba and others carry the organism without developing the disease? Do you believe there is something in the nature of bowel function which determines the course?

DR. WILLIAM J. GRACE: There are some observations that indicate a relationship between the character of the intestinal tract and the

susceptibility to symptoms in amebiasis. We have had a few patients in whom symptoms of amebiasis developed only during periods of stress. It seems to be a fact that the condition of the mucosa of the gastrointestinal tract determines the ability of organisms to penetrate. Norris and Rappaport made some experiments along that line. They gave an irritant enema of turpentine. Under ordinary circumstances 10 per cent of the animals could be infected, whereas under these unusual conditions the infection rate went up to 50 per cent. The observations that we have on changes in the mucous membrane under the reaction of stress indicate that the membrane becomes more fragile, and that some alteration in mucous formation takes place. It is my notion that this is the kind of condition most likely to lead to infection by an organism which otherwise might prove innocuous.

DR. READER: Do you have any opinions on this point, Dr. Kean?

DR. KEAN: Attempts have been made to increase the rate of infectivity in experimental work by irritating the intestinal tract with croton oil and there is some evidence that the rate of infectivity may be increased in this way. You might be interested in the relationship between mucous colitis and amebic dysentery. This is not directly an answer to Dr. Reader's question but is related to it in that it has to do with the state of the mucous membrane and its susceptibility to infection. The matter stems from some army experience. A number of individuals are in Veterans' Hospitals or are drawing disability pay who have been diagnosed as having amebiasis but who actually have ulcerative colitis. What happened in the past was that the patient came in with symptoms of colitis, amebas were found and thorough treatment instituted. Later recurrence occurred and was called amebiasis. The patient was again treated and later had another recurrence. This last episode which was called a recurrence was no longer, however, amebiasis but ulcerative colitis. Now the stool no longer showed parasites. Does this mean that amebiasis produced the picture of ulcerative colitis? Does this indicate that the patient had potential susceptibility to ulcerative colitis and that an appropriate trigger brought it out, in this case the attack of amebiasis? There are those who believe that in ulcerative colitis bacillary dysentery is the trigger. One must, of course, consider the fact that if 10 per cent of the population has amebiasis there are a certain number of cases in which the two diseases overlap.

DR. GRACE: We have seen a few cases in our clinic of the kind that Dr. Kean has described, namely, individuals who showed all the characteristics of ulcerative colitis and in whom the ameba was found in the stool. It was my belief that the ameba was just an incidental finding.

VISITOR: I do not think you said very much about the hepatic abscess. You did mention ten days of treatment with emetine in the case of hepatic abscess. Could we hear more about this?

DR. KEAN: The reason I did not go into it is that the subject of this conference is the treatment of intestinal parasites. We believe the evidence is fairly strong that the only hepatic lesion in amebiasis is a localized abscess and that amebiasis does not cause a specific diffuse hepatitis. This view is held despite the numerous articles to the contrary. Any enlargement of the liver, tenderness and changes in liver function tests which are thought by some to be caused by diffuse amebic hepatitis are manifestations of non-specific changes in the liver seen in any ulceration of the intestinal tract. For the treatment of amebic abscess there are two effective drugs, one is emetine and the other chloroquine diphosphate. Emetine has been the standard drug for many years. A course of treatment is ten daily intramuscular injections of 1 gr. each (65 mg.). After a rest period of three or four weeks the course of treatment may be repeated. For the past five years, or possibly a little longer, chloroquine diphosphate, introduced by Conan, has been in use. Employment of this drug is based on knowledge derived from studies of malaria, which showed that chloroquine was concentrated in the liver. It seems to be just as effective as emetine. The dosage schedule has not been completely worked out. It appears that 1 gm. the first day and 0.5 gm. daily for the next two weeks is a reasonably satisfactory program. It is a cumulative drug but its toxicity is not high. At the present time we do not yet have the courage to depend upon this drug alone and so we use emetine and chloroquine in alternating courses. Dr. Conan has had very good results using chloroquine alone.

DR. GOLD: Has terramycin any value in amebic abscess?

DR. KEAN: Yes and no. Terramycin sometimes brings about a spectacular drop in the temperature and, in the signs of intoxication,

chills and general malaise. Approximately one-third of patients with amebic abscess develop a secondary infection and the invaders are usually the colon bacilli. Terramycin produces its spectacular effect by acting on these, but it will not destroy the amebas and hence this striking change in the picture is only a temporary one. There is no objection to using terramycin in conjunction with emetine and chloroquine.

DR. READER: Perhaps we should go on to the next group. Would you say a word about the flagellates, Dr. Kean?

DR. KEAN: I think we can dispose of these very quickly. Giardia lamblia is treated by a standard therapy that is effective. There is no alternative. The drug is one which was used in malaria, quinacrine hydrochloride or atabrine®. In the usual schedule one gives five tablets of 0.1 gm. each the first day (that is, 0.5 gm.) and then three tablets or 0.3 gm. in divided doses daily for a week or ten days. The cure rate is about 90 per cent. After a week or ten days the course can be repeated. There is some debate on the question as to whether G. lamblia causes disease. There is fairly good evidence, however, that about half of the individuals who have this parasite are subject to bouts of diarrhea, symptoms of dyspepsia, atypical gallbladder disease, peptic ulcer, or so-called gastric neurosis. I shall not forget the aged dowager who developed severe colitis late in life. It was believed that she had a malignancy. She was ill for about six or eight months but would not go to the hospital and would not allow herself to be thoroughly examined. It was finally decided to try to do a barium enema in her home. All the equipment was brought there and two very competent radiologists made the examination. No malignancy could be demonstrated at that time. About six more months elapsed without results, when someone thought it might be a good idea to examine the stool. G. lamblia were found in great numbers. On the third day of quinacrine therapy the disease was brought under control. This is an exaggerated case but it helps to point out something which is worth stressing. It is disturbing to contemplate how extensive a medical work-up will be done sometimes before a stool examination is made. Fortunately, that is not so in this institution.

We might now go on to the subject of tapeworms. I like to talk about this because there has been some advance in the treatment of these worms in the last few years. The classic drug in the treatment of tapeworm is oleoresin of aspidium. It was used by Theophrastis in 300 B.C. and it has been a very effective agent against the tapeworm. However, it has a fairly high order of toxicity and has been largely replaced by quinacrine hydrochloride (atabrine) as the drug of choice. In view of the effectiveness of quinacrine and its much lower toxicity, as well as its applicability to all the common types of tapeworm, it would in these days be difficult to defend the use of the older remedy, oleoresin of aspidium.

The mode of administration of these drugs is a decisive factor. The parasite is attached by a tiny, hardly visible head to the mucous membrane of the duodenum. The problem is to get a high enough concentration of the drug to act at the site to which this head is attached. Neither of these drugs actually kills the parasite. They so affect the nervous system of the parasite that the head releases its hold on the mucous membrane. Thus the entire parasite with its head moves past the duodenum into the small intestine and beyond into the large bowel, where it no longer can take hold. The medication should be administered at a time when the intestinal tract is as empty as possible. The patient is instructed to have anything he wants except alcohol until noon. If possible it is best to skip dinner or supper. If he then takes a saline cathartic that night, we may presume that the intestinal tract or at least the upper portion of it is reasonably empty by morning. The medication is given in the morning instead of breakfast. There are those who give quinacrine in tablet form by mouth. The patient swallows a tablet every two minutes until all ten have been taken, making a dose of 1 gm. The drug is quite irritating to the gastric mucosa and frequently a good portion of the dose is regurgitated. The cure rate by this method is only about 70 per cent.

We prefer the method of transduodenal intubation for administering the medication. A duodenal tube is passed. It is possible to control its position by means of the fluoroscope but that is not necessary. If the tube is down to a level where a little bile can be aspirated, we can assume we are in the appropriate region, although it may not be actually in the duodenum. The quinacrine is then introduced into the tube. Two hours later the patient receives another dose of magnesium or sodium sulfate. The batting average by this technic is quite high, the

cure rate being about 90 per cent. On a second trial by the same procedure one is likely to get almost every case. We have recently seen five or six cases in which oral administration of the drug failed but direct introduction of the quinacrine into the duodenum produced a prompt cure. This is logical because by the method of transduodenal intubation a high concentration of the drug is brought directly to the point where it is needed and none of it is lost through vomiting. There is a practical point about the preparation of the quinacrine which is worth mentioning. The drug is relatively insoluble in water and one may spend the entire morning getting it to disintegrate. It is our practice to put the ten tablets into water in a medicine glass and allow them to soak over night to give them sufficient time to disintegrate. This results in a partial solution and suspension which can be fairly easily introduced into the duodenal tube.

If for any reason you do not wish to use quinacrine, oleoresin of aspidium can be applied in the same way through the tube. Mix 6 to 8 gm. of the freshly prepared drug with about 30 cc. of acacia and 30 cc. of a solution of magnesium sulfate. This rather vile looking concoction is put down the duodenal tube. The results are about as good with this drug as with quinacrine but because of its toxicity one is put in the position of having to defend the use of aspidium.

DR. GOLD: This is, then, really a one-dose treatment, is it not?

DR. KEAN: Yes, a one-dose treatment.

DR. ALMY: Can any other saline cathartic be used or is magnesium sulfate still considered the best?

DR. KEAN: Magnesium or sodium sulfate is usually advocated.

DR. GOLD: Sodium phosphate is much more pleasant. Would it be all right to use that instead? Taste, of course, does not matter when the material is introduced into the duodenal tube.

DR. KEAN: I see no reason why phosphate of soda would not do just as well.

DR. ALMY: Have you ever seen any toxicity from magnesium sulfate?

DR. KEAN: It may well have occurred unnoticed. Of course, the dose we use is not much greater than patients have been accustomed to taking in the form of Epsom salts or Glauber's salts. I do not imagine an important degree of poisoning is very common.

DR. READER: Are there any other questions? Dr. Gold, have you one?

DR. GOLD: I gained the impression from what Dr. Kean said at the beginning that 90 per cent cure rates reported in initial experiences with various drugs in the treatment of parasitic diseases usually dropped to about one-third with larger experience. Now Dr. Kean points to a method of treatment which involves the use of three agents in a particular regimen and he obtains cure rates of 90 per cent or so. Is there a chance that the new treatment may also let us down and in time show much lower cure rates? I mention this point not because I have any reason for doubting the superiority of the new method but because of the fact that it seems to be characteristic of the problem of therapy in the parasitic diseases that initial responses to therapeutic agents give rise to the appearance of a cure which turns out to be more in the nature of some sort of temporary suppression rather than a cure. That kind of phenomenon could operate through all kinds of treatments and produce similarly misleading results.

DR. KEAN: It is quite possible that my figures for cure rates may be reduced in three or four years. I doubt that it is likely to fall in the case of the treatment of tapeworm because the proof of the cure is the finding of the head in the stool. That leaves no room for dispute.

DR. READER: It would be nice to hear from you now, Dr. Kean, on the subject of pinworms.

DR. KEAN: There are a great many drugs which have been used in the treatment of pinworm and it has not been easy to ascertain their usefulness. Pinworm presents a very special problem. There is no cycle that is simpler than the pinworm cycle. The child or adult consumes the eggs and the adult worms develop in the large bowel. The female migrates usually at night to the perianal region and deposits its eggs there. The patient scratches the anus, contaminates the fingers with eggs, and then introduces the eggs into the mouth, thus completing the cycle. There has been a good deal of discussion about the wide distribution of pinworm eggs. They have been found in strange places, on chandeliers and under carpets, but if those locations were of any importance we would probably all have pinworm disease. The factor that keeps the disease going is the scratching of the anus and the subsequent introduction of the eggs into the mouth. Anus scratching is a very common practice. It is done by about one-

third of children and many adults. If you were to paint the perianal region with methylene blue you would be surprised to see how often dye will be present on the fingers in the morning. Or dip the fingers in the morning in a culture medium and see how often colon bacilli will grow out.

In order properly to evaluate a therapeutic agent in the treatment of pinworm it is necessary to ascertain first what can be accomplished by stopping the child from scratching at night. If this measure were successful the cure rate for pinworm would approximate 100 per cent. The adult female of the pinworm lives only eight weeks; hence if reinfection can be prevented the disease will be cured in eight weeks. It is difficult to determine the value of drugs in pinworm because studies fail to distinguish the role of drugs and hygienic measures. Both have usually been employed together.

Hygienic measures are the most important part of any regimen of treatment. The first thing to do is to try to control the itching. An antipruritic ointment of one kind or another is applied. For a long time the ointment of ammoniated mercury was used. We prefer a preparation called perazil® ointment which contains an antihistaminic and a surface anesthetic. There must be a great many others that are just as good. We have made use of the seal-in type of pajama, a one-piece garment zippered to the top which can be tied at night. A pair of underpants is also worn. The rear is so sealed that the child must take off the garment in order to use the facilities. Now the child can attempt to scratch as much as he wants. The urge to scratch the anus is irresistible. Dr. Hall photographed children with pinworm disease after having them tied up so as to try to prevent scratching. He found that when the hand could not be brought to the anus, the anus was brought to the hand by a process of bodily contortion which was almost unbelievable.

If, then, the child is sealed in by this method and an ointment is used to allay the itching, the outlook for cure of pinworm disease is, I would say, about 50 per cent. This figure applies to adults as well. I believe that a great many of us have had pinworm disease and have cured ourselves simply by hygienic measures, by washing the hands after anal scratching.

Now, how about the use of drugs? As I have already stated, there are a great many drugs that have been employed. Hexylresorcinol is quite effective but its toxicity is too high for use in a disease so benign. Terramycin, gentian violet and piperazine citrate are the drugs of choice at the present time. The pinworm is a parasite too tough to kill. Terramycin simply deforms the eggs so that a certain percentage lose their viability. We do not know quite how piperazine citrate acts. Gentian violet appears to have some effect on the adult worm as well as on the eggs. What we aim to do is to establish a cycle of biologic attrition in which more of the parasites die off than are acquired over a particular period of time. That seems to be the best that can be done. As in the case of other parasites the cure rates in pinworm disease also vary considerably. Dr. Harold Brown at Columbia obtained cure rates as high as 85 per cent with piperazine citrate. Our own results with this compound are only about 50 per cent. It is easy to understand the higher cure rates in a well planned experiment in which all the details of treatment can be checked and controlled. The cure rate falls off considerably when the drug is used under the more usual clinical conditions. Our practice is to use all three drugs: terramycin for three days, gentian violet for seven to ten days, and piperazine for seven to ten days. We have stopped using diphenan.

DR. GOLD: I wonder what the results would be if one of these drugs were used alone for the longer period of time, I mean the length of time taken for treatment with the three drugs in succession.

DR. KEAN: That is being tested out. It cannot, of course, be done with gentian violet because toxic symptoms appear too early. We are now trying several different time schedules with piperazine. Terramycin has been tried in fairly long treatment periods but that proved unsuccessful because even while the terramycin was being given, the eggs reappeared. Furthermore, in the case of this drug there is also the question of toxicity and expense.

VISITOR: An article appeared last year in the Texas State Medical Journal in which it was mentioned that the cure rate with gentian violet and hexylresorcinol was about 70 per cent, whereas with a regimen of garlic it was over 80 per cent. What do you think about that, Dr. Kean?

DR. KEAN: I think you are referring to the article by Killingsworth and others. Garlic has been used for a long time in Europe. It may well have some effect. I do not know the pharmacology of garlic. Is it inert?

DR. GOLD: There is a good deal of potency in garlic, aside from the odor. It contains toxic sulfides and aldehydes as well as many other substances, and it would not be safe to regard garlic as a placebo in any of these studies. How useful garlic is, however, in any of the conditions for which it has been used, still remains to be shown.

DR. READER: Is there anyone who has any questions he would like to put to Dr. Kean?

VISITOR: What are the doses of these drugs for pinworm?

DR. KEAN: In the case of terramycin 1 gm. a day is given to adults and proportionately lower doses to children for three days. The adult dose of piperazine citrate is 0.5 gm. three times a day for seven to ten days. It is not at all toxic so that the same dose could be used for children but it is usually recommended that children under the age of five receive 0.1 to 0.2 gm. three times a day. The dose of gentian violet for adults is 1 gr. (65 mg.) there times daily. The daily dose for children is $\frac{3}{20}$ gr. (9 mg.) for each year of age. A child of six would receive two tablets three times daily, each tablet containing $\frac{3}{20}$ gr. (9 mg.).

DR. GRACE: What hygienic measures do you recommend for children?

DR. KEAN: A lot depends on the harassed mother. The ideal course would be to wash and iron the pajamas daily. They do not need to be sterilized—washing and ironing are sufficient. We generally compromise and insist only on having the underpants washed and ironed daily, the pajamas twice a week. One hygienic measure which I consider absolutely crucial is the shower in the morning, not at night, because it is in the morning that you wash away the eggs which are deposited during the night. This is not so easy to accomplish for mothers seem to be averse to giving the child a shower in the morning and then exposing him to the elements. If there are several children in the same household, the difficulty of getting this done is compounded. The fact remains that a shower at night, while it may be good for other purposes, is not any good for the treatment of pinworms.

DR. READER: What about enemas?

DR. KEAN: There is considerable demand for enemas in the treatment of pinworm. The mother thinks that something is really being done when a pinworm or two is washed out. The fact is that enemas have never proved successful in curing pinworm no matter what has been put in the solution. There are also psychologic reasons for not giving enemas to a child every night of the week. This consideration might be set aside if only the enemas were effective, but they are not.

SUMMARY

DR. B. B. ROY: Parasitic diseases of the intestine are numerous and present a variety of problems in regard to incidence, clinical types, economic groups in the population and geographic situations. Indeed the problems of treatment envisage more than the choice of a remedy. The most efficacious therapy is not necessarily the one suitable for adoption in a particular area or instance. Considerations include toxicity, ease of administration, cost and length of medication, and the patient's ability to cooperate in a therapeutic regimen. It can hardly be expected to cover the entire field in one session.

Dr. Kean wisely selected for discussion a few of the diseases commonly encountered in the New York City area. However, these are not peculiar to this city but are widely prevalent throughout the world, and this fact has added wider significance to the discussion. This session has been profitable in developing the perspective necessary for an appreciation of the general problems of treatment of parasitic infections of the intestine.

A long list of drugs is available for therapy in intestinal infections, some old, some new. The point was made that reported results of various treatments should be regarded with suspicion, inasmuch as initial evaluation seldom presents the ultimate facts. In intestinal amebiasis, because of unsatisfactory results with individual drugs, the treatment plan discussed includes a combination such as emetine, an antibiotic and an arsenical; additionally, in cases of recurrence, a halogenated hydroxyquinone derivative. Details regarding choice of drugs, dosage, length of medication and related problems were outlined and discussed. There was considerable discussion regarding the course to be adopted in cases of asymptomatic amebiasis. The consensus of the Conference was that in view of the public health aspects of the problem and the potential danger of hepatitis such patients should be treated wherever practicable using the same plan suggested for acute amebic dysentery. It was pointed out that these patients who thought they were in good health felt much better after a

course of therapy. Other items of interest included the comparative efficacy of aureomycin and terramycin, the role of stress in precipitating attacks of amebic infection by inducing changes in intestinal mucosa and the relationship, if any, between amebiasis and ulcerative colitis, as well as the question of whether cysts formed in the intestine of a host can divide and release trophozoites without crossing the gastric barrier of a new host. In hepatic amebias s the lesion is a localized abscess and not a diffuse hepatitis. Recently chloroquine has shown promising results but perhaps it is not yet safe to use it to the exclusion of emetine. The effect of terramycin, which is sometimes spectacular, is related to eradication of associated bacterial infections, not to any specific action on amebas

Atabrine, now rarely used as an antimalarial, has recently found two useful applications in the treatment of infections caused by G. lamblia and tapeworm. In the former it is the standard drug. In the latter it is at present preferred to the classic remedy, oleoresin of aspidium. The problem in tapeworm infections is to get a high concentration of the drug to act at the site to which the head of the worm is attached. The mode of administration in this disease is therefore a decisive factor. Details of preparation of the patient, administration of medication and evaluation of the results were fully discussed. In pinworm infection the importance of hygienic measures has been emphasized. Since the life of the female worm is about eight weeks, prevention of reinfection for this period could eradicate the disease by hygienic measures alone. However, it is current practice to combine hygienic measures with drugs. It is noteworthy that enemas, a time-honored favorite, are considered ineffective no matter which drugs may be employed.

Clinico-pathologic Conference

Rheumatic Heart Disease, Auricular Fibrillation, Fever, Hematuria without Anemia, Splenomegaly and Sudden Death

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A TWENTY-SEVEN year old white male salesman (No. 108212) was admitted to the Barnes Hospital on June 23, 1954, complaining of severe dyspnea.

The patient had been in good health until the age of nine years when a febrile illness developed characterized chiefly by painful swollen knee joints and two episodes of epistaxis. Diagnoses of acute rheumatic fever and rheumatic heart disease were made at the St. Louis Children's Hospital. He was sent to a children's convalescent home where he remained for two years without apparent symptoms of rheumatic activity. He remained at bed rest for two or three months after returning home.

Despite the knowledge that he had a "bad heart," the patient was able to resume full activity during the next twelve years with only a few restrictions. He finished high school, avoiding all athletic activities except for occasional swimming. He did not complain of dyspnea except on marked exertion.

One year before admission his physician noted irregular heart action and prescribed digitalis which the patient took regularly until admission to this hospital. Four months before admission he had a "wisdom tooth" extracted, receiving no medication before or after the procedure. Six weeks later he noted the onset of dyspnea on moderate exertion and sleeplessness. Shortly thereafter attacks of sudden breathlessness occurred which would awaken him at night. These symptoms progressed until he was forced to quit work a few days before admission because of dyspnea on minimal activity, marked weakness and inability to sleep except in the sitting position. He was referred to this hospital for evaluation.

His mother and two siblings were in good health, although the mother allegedly had had "rheumatic fever" in childhood. The father and his three sisters all had "goiters." The patient's personal and social histories were non-contributory.

Physical examination at the time of admission revealed a temperature of 37.5°C., pulse 72 with irregular rhythm, respirations 24 and blood pressure 158/60. The patient was a thin, chronically ill white man in no acute distress at rest. The skin was warm and moist with no petechiae. Marked palmar erythema was noted. No "splinter" hemorrhages were seen. There was no significant lymphadenopathy. The optic fundi were normal and a definite lid lag was present. Several definite petechiae were seen on the hard palate. The thyroid gland was not enlarged. Except for a slight precordial bulge the configuration of the chest was normal. Examination of the lungs was not remarkable. Inspection of the precordium revealed a diffuse point of maximum impulse in the sixth intercostal space at the anterior axillary line. A thrill was palpable in diastole over this area. The area of cardiac dullness was greatly increased, extending from the left anterior axillary line to 3 cm. to the right of the sternum. A grade IV high pitched decrescendo diastolic murmur was heard along the left sternal border. Over the aortic area a grade II harsh systolic murmur was present, radiating toward the neck vessels. A grade IV harsh systolic murmur was heard over the cardiac apex. A sharply localized grade III

rumbling murmur heard throughout diastole was present at the point of maximum impulse. The cardiac rhythm was totally irregular without pulse deficit. The patient's head nodded perceptibly with cardiac systole, a capillary pulse was present and "pistol-shot" sounds were heard over the femoral arteries. Liver dullness was encountered 2 cm. below the right costal margin. The spleen was easily palpable 4 cm. below the left costal margin. No pretibial edema was present. Neurologic examination was within normal limits.

The laboratory data were as follows: red blood cell count, 5,630,000; hemoglobin, 13 gm. per cent; white blood cells, 4,750; differential: basophils, 2 per cent; stab forms, 2 per cent; segmented forms, 65 per cent; lymphocytes, 25 per cent; monocytes, 6 per cent. Platelets appeared adequate in number. Urinalysis: specific gravity, 1.020; reaction, 6.5; protein, 3 plus; sugar, negative; microscopic, 10 to 20 red blood cells per high-power field. Blood cardiolipin: negative. Blood chemistry: nonprotein nitrogen, 23 mg. per cent; total proteins, 5.9 gm. per cent; albumin, 3.9 gm. per cent; globulin, 2.0 gm. per cent; protein-bound iodine, 4.9 μg. per cent; cephalin cholesterol flocculation, negative; thymol turbidity, 0.9 units. Roentgenogram of the chest: marked cardiac enlargement with left auricular and left ventricular enlargement; rheumatic heart disease with mitral stenosis and aortic insufficiency. Electrocardiogram: slow auricular fibrillation, left ventricular enlargement and digitalis effect. Culture of the throat: heavy growth of neisseria; few beta-hemolytic streptococci. Antistreptolysin "O" titer: 100 units. C-reactive protein: 1 plus. Sedimentation rate: 7 mm. per hour, corrected.

Shortly after admission seven blood cultures were drawn. Over the next three weeks these cultures revealed no growth and were discarded. On the second hospital day the patient was given antibiotic therapy with aqueous penicillin 10 million units per day, and streptomycin 0.5 gm. every six hours. Throughout the hospital course the patient was maintained on a 2 gm. salt diet and digitalis leaf 0.1 gm. per day, by mouth. On the third hospital day temperature rose to 38.5°c., and fluctuated between 38° and 39°c. for the next five days. During the first ten days in the hospital the patient remained comfortable, complaining only of pain at the site of the needle injections. During this period eight microscopic examinations of the urine revealed between 10 and 50 red blood cells per high-power field; the sedimentation rate rose slightly to 18 mm./hour, corrected. After the seventh hospital day temperature did not rise above 38.5°c., but reached a level of 38°c. daily.

On the twelfth hospital day generalized urticarial wheals developed which were attributed to the penicillin therapy. Penicillin treatment was discontinued for about twenty-four hours, and urticaria disappeared during pyribenzamine® therapy. The previous penicillin regimen was resumed the following day and the patient had no further reaction. About this time many more petechiae were noted on the hard and soft palate, although none were seen elsewhere. The patient continued to be free of respiratory distress. The spleen continued to be easily palpable.

During the fourth week of hospitalization the antistreptolysin "O" titer was 100 units; the C-reactive protein test was negative and the white blood cell count was 14,900 per cu. mm. Low grade fever and moderate tachycardia continued but the patient remained free of any distress.

Six days before his death penicillin therapy was discontinued. The following day four blood cultures and one bone marrow culture were obtained. These cultures had revealed no growth at the time of the patient's death. Four days before his death the patient's temperature fell to levels below 37.6°c. and remained below this level. On the thirty-first hospital day the patient was found lying across the bed gasping for breath with a thready, rapid pulse. Blood pressure was not obtainable and cardiac action ceased within a few minutes on July 27, 1954.

Dr. Edward Reinhard: The patient we are considering was a twenty-seven year old white salesman who was admitted to Barnes Hospital on June 23rd with the chief complaint of dyspnea. He had been in good health until the age of nine years when a febrile illness developed characterized chiefly by painful, swollen knee joints and two episodes of epistaxis. This illness was diagnosed as acute rheumatic fever and rheumatic heart disease. Three months prior to admission the patient had a "wisdom tooth" extracted, receiving no medication before or after the procedure. Six weeks later he noted onset of dyspnea characterized by sleeplessness, shortness of breath and attacks of nocturnal breathlessness. On physical examination his temperature was slightly elevated, pulse was

normal in rate but with an irregular rhythm, respiratory rate was increased and he had slight systolic hypertension with increased pulse pressure. There was a grade IV harsh systolic mitral murmur and a diastolic mitral murmur which was sharply localized to the point of maximum impulse, of grade III intensity, and rumbling throughout diastole. Dr. Massie, we may assume that this patient had rheumatic heart disease. Would you specify the valvular lesions?

DR. EDWARD MASSIE: With respect first to the aortic valve, a loud systolic murmur at the aortic area and a diastolic murmur at that area and along the left sternal border would be accounted for by aortic stenosis and aortic insufficiency. Concerning the mitral valve, an apical systolic and a diastolic rumbling murmur would indicate that the patient had mitral stenosis and mitral insufficiency. Of course if we did not suspect rheumatic heart disease so strongly, one might consider the diastolic murmur at the apex an Austin Flint murmur. I am sure, however, that the patient had aortic stenosis, aortic insufficiency, mitral stenosis, mitral insufficiency and auricular fibrillation.

DR. REINHARD: The house staff did not diagnose aortic stenosis. That is a question of interpretation of the significance and intensity of this murmur and is hard to determine from reading a chart. It is easier to tell when you have heard the murmur. Is that not correct?

DR. MASSIE: That is right. Of course, if one had a systolic thrill at the aortic area with a loud murmur as described here one would have incontrovertible evidence of aortic stenosis. I believe, however, that with a rheumatic history, a systolic murmur at the base and an associated aortic insufficiency there should be some concomitant aortic stenosis.

DR. REINHARD: It was stated in the protocol that the rhythm was grossly irregular, from which we conclude that the patient had auricular fibrillation. The first five electrocardiograms are worthy of comment. Dr. Paine has seen the entire set of records.

DR. ROBERT PAINE: These tracings bear out the diagnosis of auricular fibrillation. The changes as reported in the protocol indicate there was left ventricular strain and probable digitalis effect. The electrocardiogram did not show anything that would verify the presence of right ventricular enlargement, but certainly the record did not exclude right ventricular enlargement.

DR. REINHARD: Let us proceed to the noncardiac findings. It was stated that the patient had marked palmar erythema. There were petechiae on the hard palate which appeared in larger numbers later on during the patient's hospital course. A definite lid lag was reported, liver dullness was noted 2 cm. below the right costal margin and the spleen was definitely felt 4 cm. below the left costal margin; temperature ranged from 37° to 39°C. and the pulse was in proportion to the fever. The laboratory findings, as you will recall, showed a white cell count of 4,750 on admission, and a little later on leukocytosis to 13,900 was noted. The differential was unremarkable. There was an albuminuria of 3 plus on admission and hematuria on twelve separate urinalyses. Two urinalyses did not show red cells. The total plasma protein was 5.9, of which 2.0 gm. per cent was globulin and 3.9 albumin. The protein-bound iodine was 4.9 and the sedimentation rate rose from 17 mm. per hour on admission to 18 mm. per hour corrected. The antistreptolysin "O" titer was 100 units on admission and had not changed four weeks later. The C-reactive protein on admission was 1 plus; four weeks later it was negative. Dr. Elliott, would you discuss the x-rays?

DR. GLADDEN V. ELLIOTT: This patient had two examinations of the chest, one on the day after entry to the hospital, another one week prior to his death. Both examinations disclosed massive cardiac enlargement, with a tremendous left ventricle and huge left auricle producing a double density along the posterior margin. The lateral view substantiated this by showing displacement of the esophagus posteriorly by the left auricle. The lung fields on both examinations were essentially clear with calcified primary complex on the left and calcified hilar nodes. The vessels were not at all prominent.

DR. REINHARD: Dr. Zimmerman, I understand that you made a study last year of the incidence of an elevated basal metabolic rate in valvular heart disease. Would you discuss the significance of this patient's lid lag? The protein-bound iodine was a little low, if anything.

DR. HERBERT ZIMMERMAN: It has been known for a long time that there are diseases associated with hypermetabolism which are not thought to be due to thyrotoxicosis. In 1947 Drs. Levine and Smith reported[1] four cases of aortic stenosis

[1] SMITH, J. A. and LEVINE, S. A. Aortic stenosis with elevated basal metabolic rate simulating hyperthyroidism. *Arch. Int. Med.*, 80: 265, 1947.

in which there were hypermetabolism, complaints of nervousness, weight loss and, in one patient, a definite lid lag and proptosis. These patients all came to postmortem examination with normal thyroids. Also, it might be added that they were all treated with Lugol's solution, with no response. Last year I reviewed the records of all patients in this hospital during the past five years having a diagnosis of aortic stenosis. There were over seventy cases, among which five patients had unexplained elevated basal metabolic rates. I do not know whether one can say with certainty that these patients did not have hyperthyroidism, but the evidence seems to indicate that this syndrome can be found in patients with aortic stenosis.

DR. WILLIAM DAUGHADAY: Lid lag is a physical sign which is very difficult to evaluate, particularly on the initial physical examination, because lid lag even in thyrotoxicosis is due to increased sympathetic tone. This is mimicked by anxiety to a great extent. Frequently lid lag is noted on our first examination of a patient who does not have thyrotoxicosis; then when we return at a later time we find that the lid lag has disappeared.

DR. REINHARD: It may not, then, have any specific significance other than as a manifestation of anxiety. We can all agree, at least, that there is nothing else to suggest that this patient had any thyroid disturbance. Dr. Rouse, we all seem to think that this patient had classic rheumatic heart disease. He had a febrile illness, petechiae, splenomegaly and hematuria. Certainly a bacterial endocarditis would have to be seriously considered. Do you believe that this patient had some type of bacterial endocarditis?

DR. ERNEST T. ROUSE: Those points you mention are in favor of it, Dr. Reinhard. The patient had one very striking negative finding, though, his blood cultures. I personally believe he did not have endocarditis.

DR. REINHARD: What was the cause of his terminal illness?

DR. ROUSE: He may well have had acute rheumatic fever, although there are many factors against that diagnosis also. The hematuria and petechiae can be features of rheumatic fever, due to the arteritis which can accompany that disease. Specifically against the diagnosis of active rheumatic fever are the serologic findings, the C-reactive protein concentrations and the antistreptolysin titers.

DR. REINHARD: Dr. Glaser, would you discuss those findings and also tell us whether you think this patient had bacterial endocarditis or acute rheumatic fever, or neither?

DR. ROBERT J. GLASER: As far as the serologic findings are concerned, I agree with Dr. Rouse that the failure of the antistreptolysin "O" titer to rise to some extent, and, more importantly, the failure of the C-reactive protein to be abnormal are both against the diagnosis of acute rheumatic fever. The latter test in particular is of value as a method of following patients. It is not a specific test of rheumatic fever but in over 90 per cent of rheumatic patients followed up for a period of time the C-reactive protein test will be positive; that really is its most valuable usage. It is not infallible, but its absence here would tend to make me reluctant to make the diagnosis of rheumatic fever. Clinically there are good reasons to assume that this man may have had acute rheumatic fever. It is said that many patients with advanced rheumatic heart disease, when they go into failure or into their terminal illness, do have a reactivation of the process.

DR. REINHARD: Even if we agree that this patient might have had a reactivation of acute rheumatic fever terminally, do you think this would have accounted for his entire terminal course?

DR. GLASER: No. It should be mentioned that acute rheumatic fever is said to develop not infrequently in patients who have subacute bacterial endocarditis. If one belongs to the orthodox streptococcal school, the streptococci that cause subacute bacterial endocarditis are not usually the ones that have to do with rheumatic fever. This patient may well have had subacute bacterial endocarditis, but I agree with Dr. Rouse that the negative blood cultures, particularly with the technic Dr. Harford has set up in our clinical laboratory, are against it.

DR. REINHARD: Two primary diagnoses have been suggested, namely, subacute bacterial endocarditis and acute rheumatic fever. Dr. Smith, do you prefer either of these two, or something else?

DR. JOHN R. SMITH: This patient displayed sufficient evidence of subacute bacterial endocarditis to justify the treatment. However, I think he died of myocardial insufficiency. I have seen patients with chronic rheumatic heart disease (with mitral stenosis) who exhibited this clinical picture in their terminal illnesses: that is, rapid deterioration of cardiac function and death within days or weeks. The

heart may be greatly enlarged. Dyspnea, sweating about the face and pallor may be striking, although systemic venous pressure may not be elevated and peripheral and pulmonary edema are minimal or absent. Fever often occurs but may disappear hours or days before death. The marked enlargement of the heart and collapsing peripheral circulation even suggest severe active carditis, although acute rheumatic lesions may not be found in the cardiac tissues at autopsy. The mechanism of death, therefore, is obscure, although the events indicate the occurrence of myocardial failure with concomitant peripheral vascular collapse.

DR. GLASER: I would like to make one other point. I think that if this man had bacterial endocarditis the pathologist may well find that the process was healed. It is well known, particularly in those patients with advanced heart disease, that many of the patients who are cured of endocarditis succumb subsequently to congestive heart failure.

DR. REINHARD: Dr. Massie, I was not quite as impressed as Dr. Smith was that the evidence summarized in the protocol signified that he had any massive and overwhelming congestive failure. Still, he may have had failure. Do you think this was the cause of his death?

DR. MASSIE: On admission the findings in the lungs were not remarkable; there was no pretibial edema and certainly, from the description we have, heart failure was not prominent. Nevertheless, it probably was present. In his final days this patient might have had a fair number of pulmonary complications, but they probably were primarily pulmonary emboli; heart failure with pulmonary emboli would account for a good part of this patient's terminal illness, including fever, without forcing us to consider rheumatic fever and endocarditis. However, I believe he had one of the latter two.

DR. REINHARD: Dr. Kingsland, does the fact that this patient had a tooth extracted six weeks before the onset of his terminal illness mean anything?

DR. ROBERT C. KINGSLAND: That was a fairly long interval, Dr. Reinhard. I do not believe you could tell when the onset of symptoms of subacute bacterial endocarditis really occurred but, in my opinion, that was his underlying disease.

DR. REINHARD: This is a very common event preceding the onset of subacute bacterial endocarditis, is it not?

DR. KINGSLAND: It is.

DR. REINHARD: Dr. Paine, does the auricular fibrillation have any influence on the incidence of bacterial endocarditis?

DR. PAINE: It has been said that hearts that are fibrillating are less susceptible to endocarditis. I believe this is true because fibrillation represents a final chapter in the evolution of rheumatic heart disease, when the valves are scarred and organisms find it difficult to grow on them. It seems to me that this patient's situation might be interpreted in a third way. This might well be the occurrence of multiple emboli from a fibrillating heart. The episodes at the end were mainly embolic. The reason the blood cultures were negative is that there were sterile emboli from the auricle.

DR. REINHARD: Would you get enough to produce large showers of petechiae in the skin and mucosa?

DR. PAINE: I have seen that happen and I am sure that the behavior of the case generally fits that pattern. We could list several things that are consistent: this patient was not anemic, sedimentation rate was not very high and cultures were negative.

DR. REINHARD: It is customary to classify bacterial endocarditis into two types, the acute and the subacute, depending upon the rapidity of the progression of the disease in the untreated case. In general the acute rapidly progressive form is supposed to be due to pyogenic bacteria, whereas the subacute type is associated with organisms of lower virulence. Dr. Jones of Iowa classifies any case of bacterial endocarditis as subacute if the patient lives more than five or six weeks.[2] This patient had an illness lasting perhaps two and one half months before therapy was started, and therefore if it was endocarditis it would have had to be subacute.

I have been impressed that staphylococcic endocarditis appears to be becoming more common. It has been pointed out that petechiae and splenomegaly are common in streptococcal endocarditis and much less common in staphylococcal endocarditis, whereas arthritis, cough, chest pain, meningitis and subcutaneous abscesses are commoner in staphylococcal endocarditis. On this basis we could agree that this man did not have a staphylococcal endocarditis. If we concentrate only on those cases of bacterial endocarditis which run a subacute clinical

[2] JONES, M. Subacute bacterial endocarditis of non-streptococcic etiology. *Am. Heart J.*, 40: 106, 1950.

course, most are due to streptococci. The commonly quoted figure is that 90 to 95 per cent of subacute cases are due to some variety of alpha-hemolytic streptococci. Dr. Wood, assuming that this patient did have bacterial endocarditis, is there any information in the protocol which would lead you to doubt that this was due to some strain of the alpha-hemolytic streptococcus?

DR. W. BARRY WOOD, JR.: No I saw the patient, as I recall, on the second or the third hospital day with the house staff and the students; at that point we had taken seven blood cultures. All agreed that this might be bacterial endocarditis and thought that treatment was indicated. There were one or two things that bothered us. The white count was low; 4,750 for bacterial endocarditis of any kind is unusual. It can occur but is not a usual count. Also, no anemia was noted and the sedimentation rate was relatively normal. Although bothered by these factors, we thought the patient should be treated. Dr. Massie has often raised the question about stopping treatment and putting the patient on salicylates. We were committed to treating this patient at the beginning and, I think, correctly. The question always arises as to how long one should maintain therapy, and I would like to ask Dr. Massie if he thinks that we treated the patient for suspected endocarditis too long. Therapy was stopped after twenty-one days.

DR. MASSIE: As a very arbitrary rule, I should advise that certainly one should treat for fourteen days.

DR. REINHARD: Dr. Wood, do you believe that the fact that this patient did not respond too well to treatment with penicillin rules out subacute bacterial endocarditis due to alpha-streptococci?

DR. WOOD: It makes me think that the patient probably did not have bacterial endocarditis, Dr. Reinhard, because most cases of subacute bacterial endocarditis due to Streptococcus viridans respond to adequate antimicrobial therapy; this patient received intensive treatment and I would have expected a good response. The patients who do not respond are those who have drug-resistant organisms, particularly in the enterococcus group. In these cases one can culture the organism from the blood, so the fact that there were no positive blood cultures plus failure to respond to penicillin leads me to agree with Dr. Paine that perhaps this condition was old rheumatic heart disease with resultant complications rather than either active rheumatic fever or endocarditis.

DR. REINHARD: In the article in the *American Heart Journal* referred to previously, Jones reviewed from the literature all the cases of subacute bacterial endocarditis in patients living more than five or six weeks, excluding all cases of streptococcal origin. There are a large number of organisms that can cause subacute bacterial endocarditis, namely, Pseudomonas aeruginosa, micrococcus, Neisseria gonorrhoeae, Neisseria meningitidis, other neisseria, brucella, hemophilus, Diplococcus pneumoniae, gram-negative rods, corynebacterium, Mycobacterium tuberculosis and actinomyces. I take it from what you said, Dr. Wood, that you believe that we do not have to consider seriously any of these.

DR. WOOD: Dr. Harford has set our procedure in the laboratory now so that in these cases thioglycolate culture is made as well as a routine broth culture. With the methods now in use we would pick up most of the organisms that Dr. Jones has listed. We would not grow the tubercle bacillus but this is a pretty uncommon cause of bacterial endocarditis; certainly we would not worry about it too much. Most of the other organisms would grow out of the media that we use, with the possible exception of the brucella organism.

DR. REINHARD: Dr. Harford, perhaps we had better go back to the blood cultures in more detail. Seven blood cultures were drawn during the patient's first twenty-four hours in the hospital before penicillin therapy was instituted. During this time the patient's temperature did not go above 37.5°C. These cultures were done routinely and by special methods. They were all negative and were discarded at the end of three weeks. On the fifth hospital day two blood cultures were obtained. At that time the patient's temperature was fluctuating between 38° and 39°C. Penicillinase was added to the cultures, which were also negative at the end of three weeks and were discarded. Five additional blood cultures, including at least one culture on a special medium for fungi, and a bone marrow culture were obtained during the last three weeks in the patient's hospital course; none of these showed any growth at the time of the patient's death. Now if this were bacterial endocarditis, what kind would you think most likely? Or do you think this was not bacterial endocarditis?

DR. CARL G. HARFORD: We cannot exclude bacterial endocarditis on the basis of negative

blood cultures; I say that not because I think that the media would not grow the organisms concerned, but because there are forms of subacute bacterial endocarditis in which the disease can be diagnosed post mortem and organisms apparently are not actually in the blood. One of the prominent reasons is that the organisms are sometimes covered over by the fibrin of the vegetation and do not get into the blood stream. It has been pointed out that the fatality rate for patients with subacute bacterial endocarditis who do not have positive blood cultures is higher than for those in whom the diagnosis can be made by this procedure. One of the reasons often given is that treatment is not instituted because a definite diagnosis cannot be made. It seems to me that it was necessary to treat this patient for subacute bacterial endocarditis because of the general clinical picture and because that disease could not be excluded on the basis of the clinical data.

DR. REINHARD: This patient was thought to be improving by the physicians taking care of him. His temperature had certainly been lower for several days when he was found lying across his bed gasping; he died shortly thereafter. Dr. Smith, what do you suppose was the cause of death?

DR. SMITH: It could be reasonably argued that massive pulmonary embolism occurred, or that a deleterious cardiac arrhythmia supervened to evoke sudden death. Heart failure may occur without peripheral vascular congestion. On the other hand, in the usual case of congestive heart failure, it would seem that the venous transport of blood from the periphery (by capillary vis-a-tergo and other propulsive forces) exceeds the capacity of the heart to accept venous blood for onward passage. Venous congestion then gradually occurs and becomes intensified by salt and water retention and resultant expansion of blood volume. However, it seems to me that in the clinical instance here myocardial failure progressed to the point where life could not be supported, but with sufficient vascular blood pooling (possibly venular) so that venous engorgement was not apparent.

DR. MASSIE: The patient had aortic stenosis in which sudden death is always a possibility; of course, the patient could have had a cerebral embolus.

DR. WOOD: Dr. Massie, would a central embolus cause a patient to die this rapidly?

DR. MASSIE: Your point is well taken. Aortic stenosis with sudden death or pulmonary embolism are the two most likely possibilities.

DR. GLASER: One other lesion, the so-called non-bacterial thrombotic endocarditis, could have produced this picture. It is postulated that some of those people have had bacterial endocarditis which is healed in the manner in which Dr. Harford suggested, and pieces of sterile vegetation break off. We have seen at least one patient in this hospital with a resistant strain of endocarditis who obtained a spontaneous cure and was a candidate for that sequence. She has rheumatic heart disease and she obviously has vegetations which are no longer infected. That is one other possibility.

DR. REINHARD: One would state that this patient might have had thrombotic endocarditis, but it would be perhaps unlikely that that would be the explanation of the whole terminal illness. Would you agree?

DR. GLASER: Yes.

DR. HARFORD: This patient did not have anemia. Could that be explained by a relatively short period of time having elapsed since the onset of a possible subacute bacterial endocarditis?

DR. REINHARD: Yes, certainly the anemia due to chronic infection does require a certain length of time to develop, depending upon the severity of the infection. A low-grade infection such as one has on the heart valve might persist for a good many months before any significant anemia would develop. That is quite possible.

DR. HARFORD: How long had the patient had symptoms?

DR. REINHARD: It is difficult to say just when the terminal illness began, but he had had symptoms for two and a half months. They were strikingly different from those symptoms he exhibited prior to that time. The house staff diagnosed the condition as rheumatic heart disease with mitral and aortic involvement, chronic auricular fibrillation, subacute bacterial endocarditis and rheumatic heart disease suspected. I think it is impossible to make a definite diagnosis here but I prefer a diagnosis of some form of subacute bacterial endocarditis. The patient might well have had multiple emboli and I believe did have multiple emboli associated with the final hours of his life.

PATHOLOGIC DISCUSSION

DR. DANIEL L. ROSENSTEIN: The most significant gross pathologic lesions were in the heart,

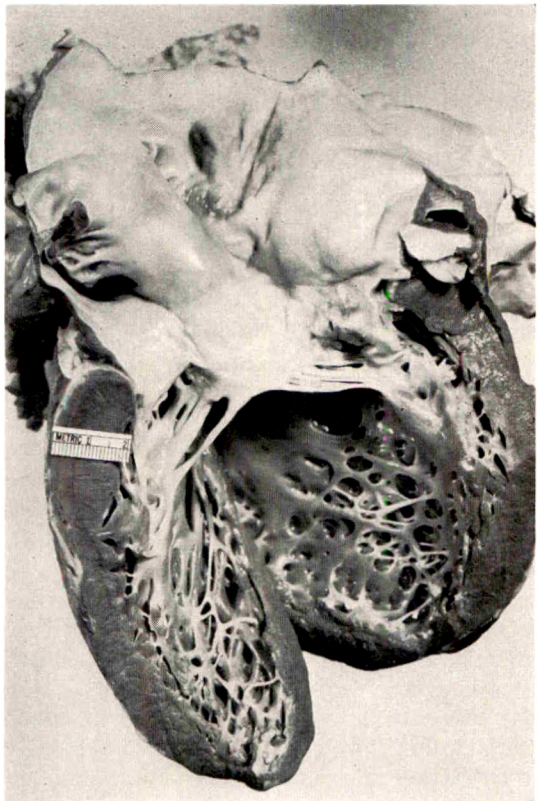


FIG. 1. The heart with left atrium and ventricle opened through mitral valve to show greatly thickened myocardium and scarring and stenosis of valve.

kidneys, spleen and lungs. The heart weighed 840 gm. and was greatly enlarged. Figure 1 is a photograph of the opened left ventricle and atrium. The atrium was dilated and thick walled and there was a patch of thickened, rough endocardium above the posterior leaflet of the mitral valve. The thickness of the myocardium of the left ventricle was 16 mm. and that of the right was 6 mm. Small scattered foci of fibrosis were grossly discernible in the posterior wall of the left ventricle. The aortic valve had thickened cusps, the edges of which were contracted and rolled toward the sinuses. There were adhesions between the cusps at the commissures, and between two of the cusps there was a lobulated calcified mass 5 mm. in diameter. The position of the cusps of this valve were such that it was undoubtedly insufficient. The mitral valve leaflets and chordae tendinae were greatly thickened and the valve moderately stenotic. The commissures were blunted by fibrous adhesions and in each leaflet there was a small calcified mass.

The kidneys were moderately congested and in the lower pole of the left kidney there was a deep sharp scar of the type that follows healing of an infarct. The spleen was enlarged to a weight of 345 gm. and was congested. The parenchyma contained prominent follicles. There was also moderate congestion of the liver and the mucosa of the upper gastrointestinal tract. The lungs were somewhat firmer than usual. Their color was tan and on incision there was minimal evidence of edema.

In summary the most significant findings were chronic passive congestion of the lungs and cardiomegaly which was predominantly left-sided with the anatomic findings of aortic insufficiency and mitral stenosis with some calcification.

DR. W. STANLEY HARTROFT: The central findings in this case are those of old scars in the endocardium, myocardium and valves of the heart with no evidence of active lesions. The myocardial scars are quite typical of those found in cases of old rheumatic myocarditis. Figure 2 illustrates such an area with fragmented ends of atrophic myocardial fibers buried in a rather loose fibrous stroma. These isolated fragments of muscle were once thought to be the source of the Aschoff cells in rheumatic nodules but are more typical of the late stages of the lesion and are now interpreted as an effect of the inflammation and scarring rather than a specific part of the inflammatory response. Figure 3 shows one of the typical, smaller and rather fusiform scars about blood vessels of the interstitial septa. This slide was stained by the periodic acid-Schiff method and the rather amorphous dark mass in the center of the illustration stained positively in the manner of the so-called mucopolysaccharides rather than like the non-specific reaction of ordinary fibrous tissue in a scar. These remnants have been said to represent the tombstones of Aschoff nodules. If so, they are the only evidence that is even suggestively specific of the rheumatic process in this patient.

Microscopic sections of the lungs show dilated alveolar spaces that are held rigidly open by turgid capillaries. This might be called pseudo-emphysema, but it is not true emphysema as the walls of the alveoli are intact. The advanced congestion of all the small pulmonary vessels and the capillaries is well shown in Figure 4 in which the erythrocytes are stained darkly. Despite the congestion there is very little

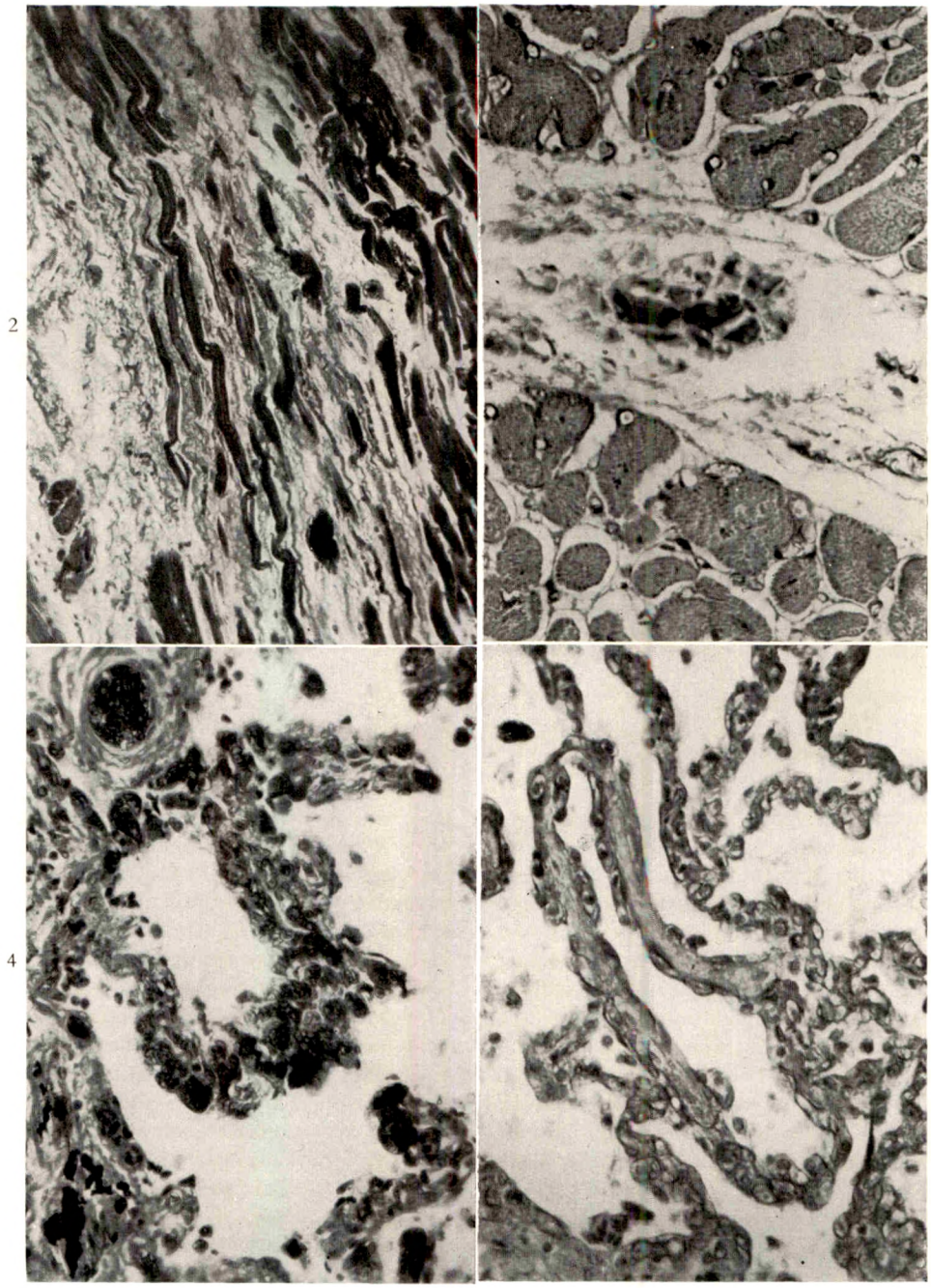


FIG. 2. Focus of fibrosis in myocardium with atrophy and fragmentation of muscle fibers. This appearance is often seen in chronic heart disease following rheumatic fever.

FIG. 3. Fusiform interstitial and perivascular nodule in the heart. Location and shape of these scars is reminiscent of Aschoff nodules and the amorphous material in the center stains with periodic acid-Schiff stain in the manner of fibrinoid.

FIG. 4. Advanced congestion of capillaries and small arteries in the lung. Turgid capillaries hold open the alveoli.

FIG. 5. Alveolus with greatly thickened basement membrane in alveolar wall.





6 7

FIG. 6. Hemosiderin-laden phagocytic cells that lie within alveolar walls as well as being free in the lumen.

FIG. 7. Advanced arteriosclerosis of small pulmonary artery that has progressed almost to point of occlusion and is thought to have originated on basis of organization of small emboli or mural thrombi.

precipitate in the alveoli to indicate the presence of a protein-containing edematous fluid. Figure 5 shows advanced thickening of the basement membrane of an alveolar septum. This is not generalized throughout the lung but is present here and there. All this material is very abnormal and where present would obviously make the interchange of gases inefficient.

In this lung, in addition, there are many macrophages which are filled with hemosiderin. A few of these can be seen peeling from the wall of the alveolus in the lower right corner of Figure 5. The origin of these cells has been debated for many years, but in this lung there are places such as that shown in Figure 6 where the cells appear actually to lie in the alveolar septal membrane suggesting that they have their origin there.

A final prominent lesion in these lungs is a widespread advanced arteriosclerosis and arteriolosclerosis. This is shown in the vessel in Figure 7 where the lumen is ridiculously small for the size of the vessel. Such vessels are almost typical of chronic mitral stenosis. Recent studies have strongly suggested that these lesions arise by organization of small emboli and mural thrombi and lead to the pulmonary hypertension found with mitral stenosis. This represents a reversal of the older interpretation that hypertension preceded the development of the vascular sclerotic lesions.

The microscopic changes in the other viscera were not very striking. In the spleen there was moderate congestion and follicular hyperplasia that could have been perhaps associated with the mild penicillin reaction experienced by the patient. The scar in the kidney had the typical appearance of a healed infarct, but the microscopic appearance of the arteries at its apex suggested it may have resulted from an arteritis associated with the rheumatic fever rather than simply an embolus. In the liver there was a suggestion of atrophy of the centrilobular cells secondary to the mild congestion present in nearly all the organs.

In summary, the pathologic changes were those of a definite chronic endocarditis with mitral stenosis, aortic insufficiency and myocardial scarring accompanied by definite but not advanced evidence of congestion in most of the viscera. These are exactly the findings that occur in the type of case to which Dr. Smith

referred. There is little evidence of the changes of advanced congestion and edema of the lungs and other viscera seen in acute myocardial failure as it is generally recognized. The changes that are present seem perfectly compatible with the physiologic explanations offered by Dr. Smith.

Final Anatomic Diagnoses: Chronic endocarditis with mitral stenosis and aortic insufficiency; hypertrophy and dilatation of the heart with myocardial scarring; chronic passive congestion of the lungs; moderate congestion of the liver, spleen, kidneys and mucosa of the gastrointestinal tract.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Research Society Abstracts

Association for the Study of Liver Diseases

ABSTRACTS OF PAPERS PRESENTED AT THE FIFTH ANNUAL MEETING, CHICAGO, ILLINOIS, OCTOBER 28, 1954

SYMPOSIUM ON JAUNDICE (MODERATOR, LEON SCHIFF, CINCINNATI, OHIO)

METABOLISM OF BILE PIGMENTS. *Irving M. London.* New York, N. Y.

Hemoglobin, myoglobin and the respiratory heme enzymes occupy a central role in metabolic processes for they are concerned with the processes by which oxygen is made available to the animal cell and with the processes of cellular oxidation. In the biologic synthesis of heme, glycine and succinate are utilized for the formation of delta-amino-levulinic acid from which a monopyrrole, probably identical with porphobilinogen, is formed. This monopyrrole can be converted to uroporphyrin, coproporphyrin and protoporphyrin.

The conversion of hemoglobin to bile pigment may proceed via the formation of choleglobin. Evidence is presented to indicate that this conversion might also proceed via hematin. In the intestine bilirubin undergoes a series of reductions to mesobilirubinogen and stercobilinogen which on oxidation yield urobilin and stercobilin, respectively. In the presence of infection, or after the administration of certain antibiotics orally, a dextrorotatory urobilirubinogen is formed which on oxidation yields dextrorotatory urobilin.

Recent studies on the direct and indirect van den Bergh reactions indicate qualitative differences in the chemical constitution of the pigments which give these reactions.

Studies on the origin of bile pigment indicate that in normal man at least 10 to 15 per cent of total bile pigment is derived from one or more sources other than the hemoglobin of mature circulating erythrocytes. In disease states such as pernicious anemia and congenital porphyria the proportion derived from alternative sources may be increased. Investigation of the nature of these alternative sources has revealed that hematin can be converted to bile pigment in the mammalian organism. Studies are now in progress on the possible conversion of other naturally occurring porphyrin compounds to bile pigment.

INTERRELATIONSHIPS AMONG HEPATIC TESTS. *Leslie Zieve.* Minneapolis, Minn.

From a study of interrelationships among liver function tests one may expect to (a) bring out relationships not apparent on the surface, (b) discover the tests having independent significance, and (c) find a rational basis for discarding tests. Such an analysis as applied to patients with acute viral hepatitis revealed that each of nine quantitative tests representative of the gamut of hepatic tests was effective in differentiating the patient with hepatitis from the normal. All nine taken together in optimal combination were no better than four of the tests taken in combination. The observed effectiveness of the five tests (galactose tolerance, thymol turbidity, per cent cholesterol esters, serum bilirubin and urine urobilinogen) not having independent significance was accountable in terms of the four having independent significance and comprising the minimal combination. These four tests were (1) bromsulfalein retention, (2) zinc sulfate turbidity, (3) hippuric acid excretion, and (4) coproporphyrin excretion. The largest contribution to the observed effectiveness of the five tests without independent significance was made by factors underlying the coproporphyrin test.

In a similar group of patients with cirrhosis, the same four tests were found to have independent significance. Unlike the hepatitis cases the largest contribution to the observed effectiveness of the five tests without independent significance was made by factors underlying the bromsulfalein test.

A simple analysis of the relationship of various tests to the degree of bilirubinemia in patients with cirrhosis, viral hepatitis and obstructive

jaundice resulted in the following observations: Percentage cholesterol esters was largely dependent on the degree of jaundice in each of the three groups. A moderate correlation between bromsulfalein retention and bilirubinemia was observed in each of the three disease groups. An inverse relationship, similar in magnitude, was noted for the hippuric acid test in the cirrhosis and hepatitis patients. With two exceptions, no appreciable correlation with the degree of jaundice was noted for the galactose tolerance, urinary coproporphyrin, urinary urobilinogen, cholesterol, thymol turbidity, zinc turbidity, cephalin flocculation and alkaline phosphatase tests. The galactose tolerance and urinary coproporphyrin tests each correlated moderately well with the degree of jaundice in the hepatitis patients only. Though the alkaline phosphatase is usually increased in the presence of obstructive jaundice, no relationship was observed between the degree of abnormality and the degree of jaundice.

HEPATITIS WITH MANIFESTATIONS SIMULATING BILE DUCT OBSTRUCTION ("CHOLANGIOLITIC" HEPATITIS). *Edward A. Gall*. Cincinnati, Ohio.

This study is concerned with the lesions observed in liver biopsy specimens from fourteen patients in whom the initial clinical course incorrectly indicated major biliary duct obstruction. This information and that gathered from clinical and laboratory observations were compared with similar data derived from thirty-five patients with classical viral hepatitis. The patients with obstructive manifestations proved to be in the older age range with more prolonged jaundice. Other clinical features were similar in both groups although those simulating obstruction more often exhibited pruritus, acholic stools, elevation of blood lipids and serum alkaline phosphatase, and negative results with the flocculation tests.

The histologic pattern varied somewhat but there was an obvious relationship between the two, and the lesion contrasted strikingly with those found in the presence of true bile duct obstruction. The prolonged ("cholangiolitic") form of hepatitis was highlighted by inflammatory predilection to periductal regions, perilobular pseudoductular epithelial budding and bile stasis. These were all present to a variable and lesser degree in the lesions of viral hepatitis as ordinarily encountered. There was no consistent correlation between individual morphologic alterations and the various features of the clinical syndrome. The problem of clinical distinction between hepatitis simulating obstruction and surgical obstructive jaundice should be resolved in most instances by recourse to needle biopsy of the liver.

SURGICAL ASPECTS OF (OBSTRUCTIVE) JAUNDICE. *Julian A. Stering*. Philadelphia, Penna.

The surgeon's approach to obstructive jaundice is influenced by the need to assure normal bile flow as a method for restoring normal hepatic function. At operation, supplementary assistance to gross inspection and palpation should be available. These include manometric methods, radiologic technics, rapid examination of removed tissues and facility for duodenotomy.

The patient's condition usually determines whether the surgeon can remove and reconstruct or merely palliate and drain. Whether bile is permitted to reach the duodenum is not as important as the relief of obstruction. Secondary surgery to reconstruct a proper route for bile flow into the intestines may be done at a later date after hepatic function has been restored.

Discussion of the Preceding Presentations and General Remarks

CECIL J. WATSON (Minneapolis, Minn.): Dr. London has given us an excellent survey of many of the aspects of the bile pigment problem. The important studies of the significance of glycine in the biosynthesis of heme and the observation that some of the stercobilin in the feces is not derived from mature circulating erythrocytes are of great interest. It was not surprising to find that this was true in pernicious anemia, as previous data had strongly indicated that much of the urobilinogen of the feces in this disease in relapse was not derived from any ordinary blood destructive process, and Whipple, in his pigment-pool concept, in addition to Minot and Jedlicka, had favored this concept. In our own studies it was repeatedly observed that the amount of urobilinogen excreted in the feces was far out of proportion to the decline of circulating hemoglobin over a given period of time, taking into account the fact that in pernicious anemia, unlike the ordinary hemolytic anemias, the rate of replacement was very slow. It was much more surprising to learn from the N^{15} glycine studies of London and his co-workers that under normal conditions about 15 per cent of the fecal stercobilin is derived from other than the hemoglobin of mature circulating erythrocytes. Here it seems that there are two main possibilities. (1) This fraction does represent pigment partially built up on the way to heme but is excreted without being used, somewhat in the sense

of the Whipple pigment-pool concept. (2) An excessive number of red blood cells are formed and at once destroyed. This would be in line with the general lavishness of compensation in excess which characterizes so many natural phenomena. Further study must decide between these possibilities.

We have recognized since our early work on crystalline stercobilin that fecal urobilinogen is not the sole bile pigment derivative excreted in the feces. At the time that stercobilin was crystallized it was regularly noted that it was accompanied in approximately equal amounts by a dark brown, almost black, substance to which the name "copronigrin" was given. This substance was characterized by an insoluble zinc complex, in contradistinction to the readily soluble green fluorescing zinc complex of stercobilin and urobilin. Siedel later showed that mesobilifuscin, a dipyrrylmethene derived from bilirubin, has these characteristics, and the isolation of mesobilifuscin from the feces by Siedel leaves little doubt that it is essentially the same as the substance earlier designated as "copronigrin." Thus it appears that some of the bile pigment from destroyed hemoglobin or possibly from other sources as mentioned in the foregoing, is represented not as a tetrapyrrylmethene of the urobilinogen type, but as a dipyrrylmethene, a molecule half as large. Much further study is necessary and is now in progress to determine the significance of mesobilifuscin and whether or not its excretion in the feces varies in a manner differing from that of urobilinogen excretion. It is conceivable that this might have considerable significance in certain states such as hepatic disease and rheumatoid disease in both of which it has recently been claimed that a shortened red cell life span may not be associated with a heightened urobilinogen excretion.

A word about the problem of the serum bilirubin. Whatever the exact basis of the prompt, direct versus the delayed or indirect van den Bergh reaction shall ultimately prove to be, it has become increasingly clear that these fractions have genuine validity in the study of disease. One of the great difficulties in determining the basis of the difference in these reactions may be ascribed to chemical artefact. It is clear that chemical treatment of one type or another of bilirubin-containing sera may alter the character of the reaction. The most recent fundamental studies of the van den Bergh reaction by Najjar in this country and by Cole and Lathe in England have given widely varying results. Najjar believes that the prompt, direct reacting type is a metal complex and the indirect type a globulin complex, while Cole and Lathe state that the indirect is simply free bilirubin and the direct a substance chemically distinct from bilirubin. They state further that bile contains relatively little bilirubin, this term being reserved for the indirect reacting type. This, however, we find very difficult to understand as Fischer showed many years ago that crystalline bilirubin from cattle gallstones is identical with the crystalline bilirubin isolated from old hemorrhages. Porsche devised an excellent method for isolating bilirubin of the same type from pig bile. Lowry and I have recently shown that crystalline bilirubin can be isolated from human feces in good yield shortly following administration of aureomycin. In addition, Snapper showed years ago that the crystals of bilirubin isolated from obstructive jaundice serum were the same as those from (indirect reacting) hemolytic jaundice serum. All of these observations indicate that the bile does contain relatively large amounts of bilirubin and, as mentioned, it has been shown to be identical with bilirubin appearing in extravasations of blood, in other words the "hematoidin" of Virchow. These comments are not meant to imply that the studies of Cole and Lathe are incorrect or without significance but it is important to question to what extent their results have been influenced by chemical manipulations, and further work is necessary in this regard. The possibility must be considered that a differing physical state of bilirubin in direct reacting sera, rather than a structural difference, influences its behavior in the chromatographic method used by Cole and Lathe.

Dr. Zieve's observations on the fundamental interdependence of certain liver functions and the emergence of certain factors common to a group of functions is believed to be of much significance. It might be pointed out that the lack of interdependence of the urinary coproporphyrin and the bromsulphalein factors in the cirrhosis group and its presence in the hepatitis group may possibly be explained by the fact that in the latter the excessive urinary porphyrin excreted in the urine is the type I isomer. There is considerable evidence that this excess is related simply to regurgitation of bile or simple hepatocellular excretory functional impairment; hence, in this instance interdependence would be expected. In the cirrhosis group, if mainly in alcoholics, as in Dr. Zieve's study, the excessive urinary porphyrin is a type III isomer. This is probably related to an intrinsic metabolic impairment, although whether in the liver or bone marrow is not yet known. Here one would expect a lack of interdependence. Further study along these lines may prove of great interest.

With respect to the problem of cholangiolitic hepatitis, I understood Dr. Gall to say that a distinction could almost always be made histologically between this condition and obstructive jaundice. If this is true his criteria will be of much help to other pathologists who are having difficulty with this problem. We have had considerable trouble in interpreting liver biopsies in this respect, and on not a few occasions the initial interpretation was eventually shown to be incorrect. Partly on this ground we have come to rely more and more on cholangiography and liver biopsy, either peritoneoscopic or through a small laparotomy under local anesthesia, in those cases where other features have not served to distinguish

cholangiolitic hepatitis or cirrhosis from extra-hepatic biliary obstruction.

It may be emphasized that we have never used the term cholangiolitic in the histologic or morphologic sense but purely from the standpoint of functional cholangiolar disturbance. When Dr. Hoffbauer and I first started using this term we were impressed by the lack of hepatocellular functional impairment in the presence of outspoken evidence of regurgitation of bile but, at the same time, without any evidence of specific or well defined lesion of the main bile passages. In these instances it seemed inescapable that when the histology is relatively normal there must be a functional disturbance of the cholangiolar system permitting a return of bile to the blood, either directly or via the lymph. It was thought, too, that this might account, at least in part, for bile thrombi, in other words an inspissation of bile due to greater loss of water than solute.

It should also be emphasized that cholangiolitic hepatitis or cholangiolar impairment is often only a part of the total picture. The situation is perhaps somewhat analogous to nephrosis and the nephrotic "einschlag" of Volhard which often accompanies glomerulonephritis to some degree. Frequently in hepatitis there is cholangiolar impairment and in some of the prolonged cases it may persist for months or years as an isolated phenomenon with relatively normal function of the liver cells. In some instances the cholangiolar system appears to be the only part of the liver which has been impaired even from the outset. Such injuries as those due to arsphenamine, methyl testosterone and, more recently, thorazine appear often to be of this type. Thus use of the term cholangiolitic hepatitis or cirrhosis should imply a marked functional impairment of the cholangiolar system with regurgitation of bile but with little or no evidence of hepatocellular functional impairment.

HISTOGENESIS OF COARSE NODULAR CIRRHOSIS. *Hans F. Smetana.* Armed Forces Inst. Pathology, Washington, D. C.

There is circumstantial evidence that coarse nodular cirrhosis is one of the possible but rare sequelae of viral hepatitis, although it may also be the consequence of toxic damage to the liver by arsenic, phosphorus or mushroom poisoning. The clinical symptoms produced by this condition are often indistinguishable from those of portal cirrhosis, and the diagnosis can be suggested only on the basis of a history of viral hepatitis. Since it may take several years for portal hypertension or hepatic insufficiency to develop, the history of hepatitis may by then be rather vague.

The gross appearance of the pathologic specimen of coarse nodular cirrhosis is characterized by replacement of the hepatic parenchyma by nodules measuring one or more cm. in diameter, which produce great deformity in the shape of the liver. Microscopically, the individual nodules are made up of composite liver lobules which are separated by fibrous bands containing reticulum fibers, fibrous connective tissue and small bile ducts. The portal triads within the composite nodules are smaller than normal and their spacing as well as their relationship to efferent veins is irregular. The liver cells are fairly normal in appearance except that their radial arrangement about efferent veins is often distorted. The reticulum of the nodules appears normal.

Histologically, the appearance of the composite nodules of coarse lobular cirrhosis is so unlike that of the pseudolobules of portal cirrhosis as to suggest an entirely different histogenesis. The pseudolobules in portal cirrhosis are single lobules and they lack a properly placed efferent vein from which columns of liver cells radiate. No portal triads are seen in the pseudolobules. It appears that the damage leading to portal cirrhosis affects groups of liver cells of each individual lobule as well as the reticulum in this area. Regeneration of liver cells therefore is irregular and often concentric. Coarse lobular cirrhosis, on the other hand, represents the healing phase of near fatal necrosis of liver tissue due to the virus of hepatitis or to toxins, which leaves only a few islets of liver cells intact. From these islets regenerating nodules of liver cells grow in size until their expansion is halted by similar neighboring nodules, while the collapsed stroma of the necrotic portions furnishes the septa. The tissue making up these composite nodules resembles normal liver tissue fairly closely except for distortion of cell columns and lack of portal triads. Reorganization is gradually accomplished by ingrowth of bile ducts from the septa into the regenerated nodules of liver cells and by regrouping of vessels. Only if enough liver cells are spared during the original attack can sufficient liver tissue be regenerated. Such regenerated liver parenchyma may function reasonably well for years but eventually difficulties in circulation within the liver lead to portal hypertension and its consequences. If anoxemia ensues because of general circulatory collapse, gastrointestinal hemorrhage, coronary disease or any other cause, the nodules may become necrotic, leading usually to hepatic insufficiency.

MASSIVE HEPATIC NECROSIS IN A YOUNG MAN. *W. Stanley Hartroft and Robert E. Shank.* Washington Univ. Medical School, St. Louis, Mo.

Clinical and pathologic findings were described pertaining to a young white man (age twenty-nine) who had manifestations of heat prostration ten days before death. At autopsy, massive hepatic necrosis was discovered. The patient had been a social worker in New York City, where he had been exposed to jaundiced people and rats, and he had occasionally worked with cleaning solutions. He had no alcoholic habits. At the height of the summer heat, he hitchhiked from New York City to St. Louis, and the next day, although tired, ran a six-mile race when the recorded temperature was 86°F. and the relative humidity was 54 per cent. Following the race he collapsed with characteristic symptoms of heat stroke, except that marked hyperpyrexia and defective sweating were absent. Laboratory data revealed evidence of hepatic dysfunction, which became increasingly severe along with jaundice during his ten-day hospital stay prior to death. His temperature on admission to the hospital was 39.2°C. Subsequently it dropped and did not go above 39°C. throughout the rest of the course, except during the terminal period. At autopsy, massive hepatic necrosis was discovered and generalized icterus of all viscera. In microsections of the small wrinkled yellow liver only narrow rims of parenchyma in periportal positions had survived. A moderate degree of chronic inflammatory reaction was present in portal triads. Pseudocholangioles had formed at the edge of the surviving tissue. Hemosiderin, hemofuscin and ceroid pigments were present in the surviving parenchymal cells and in the Kupffer cells. Little stainable fat could be demonstrated except in the walls of hepatic arterioles, where it presented an unusual and striking appearance. Microscopic observations of relevant interest in other organs were confined to the demonstration of hemorrhages in the dentate nuclei of the brain during the terminal period. Although the lesions in this case might be compatible with published descriptions of hepatic necrosis due to heat stroke, the patient's history and his course in the hospital, as well as the anatomic findings, appear more compatible with a diagnosis of infective hepatitis of viral origin. The possibility was raised that massive hepatic necrosis in heat stroke may sometimes have an infective origin characterized by a fulminating course precipitated by the superimposed stress induced by hyperthermia.

JAUNDICE ASSOCIATED WITH ADMINISTRATION OF CHLORPROMAZINE. *Joseph M. Gambescia, Joseph Imbriglia, Peter Galamage and William Winkelman, Jr.* Hahnemann Medical College, Philadelphia, Penna.

Five cases were presented of persons developing an icteric state following administration of chlorpromazine. The clinical picture was characterized by an acute febrile response with malaise, anorexia, chills, myalgia, followed by dark urine, pruritus and jaundice. The biochemical pattern in all cases showed hyperbilirubinemia, choluria and hyperphosphatasemia associated with normal cephalin-cholesterol flocculation, normal thymol turbidity and flocculation, normal zinc turbidity and serum protein partition. In some cases an eosinophilia (up to 26 per cent) and hypercholesteremia was noted. Needle liver biopsy specimens revealed intrahepatic cholestasis with a lesion affecting the cholangiole characterized by eosinophilic infiltration.

Certain therapeutic approaches were discussed. The use of antihistamine and sodium dehydrocholate had no beneficial effect. ACTH and cortisone were accompanied by prompt beneficial response. No fatalities have been encountered.

The entity is of considerable importance because of (1) its resemblance to viral hepatitis with its accompanying public health implications; (2) its resemblance to extrahepatic obstruction and its surgical implications; (3) its morbidity and its economic implications.

INTRAHEPATIC OBSTRUCTIVE JAUNDICE IN INFANTS. *Ruth C. Harris.* Columbia Univ. College of Physicians and Surgeons, New York, N. Y.

During the past few years intrahepatic disease has come to be recognized as a relatively frequent cause of obstructive jaundice in infants under six months of age, occurring with even greater frequency than does congenital atresia of the bile ducts. In cases of intrahepatic biliary obstruction the syndrome usually follows and often blends with the clinical picture of jaundice of prematurity or with that of erythroblastosis fetalis and occurs as a rule in patients who have been treated either by exchange transfusion or by simple transfusion. Similar cases observed by others have been reported in the literature as examples of viral hepatitis, while additional

instances have been included in the "inspissated bile syndrome." In our own case material, a large proportion of infants so afflicted have been of Italian parentage. Clinically these infants usually reveal persistence or recrudescence of jaundice present in the neonatal period, with the appearance of light-colored stools and dark urine. They may or may not appear ill but generally show some retardation of growth and may develop rickets during the course of the disease. The liver and often the spleen are enlarged and firm. The total serum bilirubin concentration may vary from 6 to 30 mg. per 100 ml., "direct" bilirubin comprising approximately one-half of the total. Cephalin flocculation and thymol turbidity tests usually give negative results. Serum alkaline phosphatase level is elevated in approximately two-fifths of the cases. Zinc sulfate turbidity is low or normal in infants with preceding erythroblastosis or with a history of prematurity but is elevated in the other cases of this group. Liver biopsy yields sections which are surprisingly alike in all of these cases. Liver cells at or near the centers of lobules are found filled with yellow, non-iron-containing pigment which appears similar to that filling the bile canaliculi. The liver cells are swollen, and many are multinucleated. The portal areas show an increase in mononuclear cells and slight proliferation of bile ducts. When a hemolytic process has preceded the development of obstructive jaundice, islands of erythropoietic and myelopoietic tissue are scattered throughout the lobules. Sometimes a few necrotic cells are found. The same histologic picture has been found in patients with a wide variety of disease pictures, such as erythroblastosis, serum hepatitis, acquired hemolytic anemia, cytomegalic inclusion disease and prematurity.

It appears that an insult to the liver during the first six months after birth results in a characteristic response, giving a picture similar histologically to that described in adults by Lucké and attributed by him to viral hepatitis. Swollen liver cells, overdistended with bile, occlude the bile capillaries, causing the obstructive phase of the disease. The inflammatory response seems minimal but the evidence of regeneration, as seen by the giant hepatic cell response, is good. The presence of many mononuclear cells in the portal areas, the myelopoiesis and erythropoiesis found in infants with this syndrome are related to the age of the patient and the need for additional blood cell formation. The mechanism of damage to liver parenchyma is quite different from that of extrahepatic atresia where hepatic cellular injury occurs in a relatively late stage of the process.

SPLANCHNIC HEMATOCRIT IN THE DOG DETERMINED FROM SIMULTANEOUSLY MEASURED SPLANCHNIC PLASMA AND RED CELL VOLUMES. *B. Cominsky, J. R. K. Preedy, R. Hays, H. O. Wheeler and S. E. Bradley.* Columbia Univ. College of Physicians and Surgeons, Presbyterian Hosp., New York, N. Y.

The hematocrit of blood in small vessels is less than that in large vessels, owing to lamination of flow. The hematocrit of the pooled total blood volume would therefore depend on the relative contribution of blood from vessels of different caliber.

With the development of a method for measuring splanchnic blood volume in the intact animal by regional retention and dilution of a tracer material, it is possible to measure the splanchnic hematocrit and thus to determine indirectly the distribution of blood within the splanchnic vasculature. Since in the dog the spleen is a large organ containing blood with a high hematocrit, measurements were carried out in both intact and splenectomized animals. In seven intact and eight splenectomized dogs anesthetized with nembutal® (30 mg. per kg.) splanchnic plasma volume (SPV) and splanchnic red cell volume (SRCV) were determined simultaneously by dilution of I-131 labelled albumin and P-32 tagged red cells, respectively. Splanchnic hematocrit (SHCT), calculated as SRCV/SRCV + SPV, was 79.4 ± 68.9 per cent of the peripheral arterial hematocrit in the intact and 71.8 ± 18 per cent in the splenectomized dogs. These figures do not differ significantly. In the determination of splanchnic blood volume, blood may be sampled from either a right or left hepatic vein. SHCT based on blood collected from the right was 73.9 ± 16.9 and from the left 77.0 ± 13.7 per cent of the peripheral arterial hematocrit, there being no significant difference between the two. These results indicate that the cell-rich blood of the spleen is not measured by the dilution technic used, and that the major portion of blood located in the splanchnic bed is located in small vessels, chiefly in the liver.

In five dogs (three splenectomized) simultaneous measurements of SPV, SRCV and SHCT from blood collected at the same time

from both right and left hepatic veins showed good agreement. This observation suggests that there is a uniform distribution of hepatic arterial and portal venous blood per unit mass of liver tissue.

PAPER ELECTROPHORETIC STUDIES OF SERUM PROTEINS IN VIRAL HEPATITIS. *Gerald R. Cooper*. Cummunicable Disease Center, Atlanta, Ga.

Specimens collected from forty-six patients with viral hepatitis have been analyzed by paper electrophoresis. The electrophoretic results were compared with seromucoid, C-reactive protein, bilirubin, cephalin flocculation and thymol turbidity values. Findings at the height of the disease in an average case showed an electrophoretic pattern with a distorted alpha-2 globulin peak, a slightly enlarged beta-globulin peak and a prominent increase in gamma globulin peak, a lowered seromucoid value, a one-plus C-reactive protein reading, and elevations in bilirubin, cephalin flocculation and thymol turbidity results. Persistently enlarged gamma globulin peaks were usually associated with continued elevated cephalin flocculation or thymol turbidity values. Six proved cases of posthepatitic cirrhosis gave abnormal results in all tests, especially paper electrophoresis.

SERUM GLYCOPROTEINS IN VIRAL HEPATITIS. *Fenton Schaffner, Arthur L. Scherbel and Ralph I. Lytle*. U. S. Naval Hosp. and Med. Research Unit No. 4, Great Lakes, Ill.

The serum glycoproteins were studied electrophoretically at weekly intervals in eleven patients with acute viral hepatitis from the first week of their illness until the time of discharge from the hospital and at the time of liver biopsy in three patients in whom viral hepatitis had persisted for three months or longer. The serum proteins were fractionated by paper electrophoresis. The strips were stained with bromophenol blue and treated with mineral oil and dioxan. Glycoproteins were determined on similar strips after staining with basic fuchsin. The serum gamma globulin was elevated early in the disease and returned to normal usually by the sixth week. High percentages of polysaccharides were associated with some of the gamma globulin elevations but in general the results were within normal limits and showed little change throughout the course of the disease. Beta globulin and associated polysaccharides remained normal. Alpha-2 proteins and polysaccharides were slightly elevated initially and became normal in one to two weeks. Albumin and alpha globulin were considered together. These proteins behaved as a mirror image of the gamma globulin while the polysaccharides dropped to low levels and returned to normal after the proteins. The total protein-bound carbohydrate appeared to be reduced, although this could not be well quantitated. The glycoprotein pattern differed from normal in that the amount of slower moving (gamma) carbohydrate increased while the more rapidly moving fraction decreased. This is in contrast to rheumatic fever in which the total glycoprotein was greatly increased, the greatest rise being in the more rapidly moving portions, especially both alpha globulins. In protracted hepatitis the albumin and alpha-1 polysaccharides were low and the gamma polysaccharides were high, although these changes were of the same magnitude as those in the acute stage. The only consistent feature histologically found in the three biopsy specimens was the persistence of hepatic inflammation. In general, it is felt that the behavior of the glycoproteins is a reflection of inflammation in the liver, as contrasted with changes noted in inflammation elsewhere.

SERUM COMPLEMENT IN HEPATOBILIARY DISEASE. *Emanuel E. Mandel and Kurt Lange*. Communicable Disease Center, Atlanta, Ga. and New York Medical College, New York, N. Y.

Conflicting reports concerning the serum complement titer in liver disease prompted its determination in twenty cases of cirrhosis (nineteen portal, one postnecrotic), fifteen of viral hepatitis and twenty-six of biliary obstruction which in sixteen patients was caused by neoplasm. The method, which gave a range of 1.8 to 3.6 units in twenty healthy subjects tested simultaneously, had previously been found by Lange consistently to yield low (less than 1 unit) or absent complement in acute glomerulonephritis and nephrosis. The range encountered in cirrhosis was 0.8 to 4.7 units (mean, 2.6), in hepatitis, 1.4 to 5.7 (3.2), in benign obstruction, 2.0 to 6.1 (4.0) and in cancer, 1.9 to 4.9 (3.7). Abnormally low complement was noted only in two cirrhosis patients dying of hepatic insufficiency. The majority of the group with cirrhosis and two of the patients with hepatitis had "extrahepatic" complications (pyelonephritis, pan-

creatitis, pneumonia), which may have counteracted a complement depression such as had been observed by Goldner and by Jordan in hepatocellular disease. Unusually high complement values encountered in all groups but especially in patients with benign obstruction presumably reflected the presence of an active infectious process. The present data fail to reveal any diagnostic utility of the test in liver disease, except for the occasional finding of complement diminution which may denote a grave prognosis.

Treatment of Fatty Liver with a High Fat Diet. *Gordon M. Mindrum.* Cincinnati Gen. Hosp., Cincinnati, Ohio.

A high fat diet was used in the treatment of eight biopsy-proved cases of severe fatty liver. The daily diet averaged 120 gm. protein, 300 gm. carbohydrate and over 300 gm. fat. Stool fats averaged 29.5 per cent dry weight for twenty-four hours. Duration of treatment averaged 4.2 weeks. All patients showed progressive clinical improvement with reduction of liver size, clearing of ascites and edema, gain in strength and improved nutrition. Liver function showed improvement with total bilirubin dropping to within normal limits. Bromsulfalein retention decreased to near normal and serum albumin increased. Serum lipid values did not vary significantly. Serial liver biopsy specimens in three patients revealed clearing of visible liver fat in four to six weeks. This evidence suggests that patients with severe fatty livers can absorb and utilize large amounts of fat.

Roentgenologic Visualization of Biliary Ducts by Intravenous Injection of a New Contrast Medium. *Raj K. Parida, Alexander J. Link, Julius Heydemann and Robert M. Kark.* Univ. of Illinois College of Med., Chicago, Ill.

The di-sodium salt of N,N-adypyl-bis(3-amino-2,4,6-triiodo)benzoic acid, a new roentgenologic contrast medium (chlorografin®), was used to study the biliary duct system in twenty-one patients. As corroborated by serial liver function studies in our patients, the toxicity of this compound is low when compared with other contrast media in use. Adequate roentgenologic visualization of the large bile ducts was obtained in fifteen of twenty-one patients following intravenous injection of the material. The six patients with non-visualization of the ducts had either severe hepatic dysfunction or air in the ducts due to previous surgery. The renal pelvis and calyces were visualized in several of the pictures, and this occurred more frequently in patients with known hepatic dysfunction.

The preparation appears to be a useful adjunct for the diagnosis of biliary tract diseases, especially for the study of intraductal pathology. Stones causing partial obstruction and strictures of the extrahepatic biliary ducts have been diagnosed by use of the dye and the observations were corroborated by surgery.

Nail Beds in Hepatic Cirrhosis. *Richard Terry.* Hektoen Inst. for Medical Research of Cook County Hosp., Chicago, Ill.

The nail beds may be divided into three zones, the half-moon, the main pink zone, and a narrow band running across the distal portion of the nail-bed, which is herein called the onychodermal band. The appearance of these three zones may be altered in hepatic cirrhosis.

On squeezing blood into the terminal phalanx, the normal main pink zone assumes a clear red color; in 'white nails' the redness is obscured by a whitish opacity similar in character to the half-moon. The opacity is in the nail-bed and not in the nail. Such white nails are not uncommon in clinical states but well marked examples occur most commonly in hepatic cirrhosis and in a few other disorders.

Normal half-moons have a barely perceptible pink tinge, but on occasion the half-moon becomes reddened. Such red half-moons, which are detectable at a distance of several feet, occur in cirrhosis although they are less common than white nails. Three patients with cirrhosis have been seen who had both red half-moons and white nails.

The normal onychodermal band is barely perceptible. It may, however, become opaque (with a faintly amber tinge) forming an easily detectable whitish band across the distal portion of the nail bed, 0.5 to 1.5 mm. in width. This appearance is rare, and only nine examples have been found by the author; of these, five were in cirrhosis.

The appearances described constitute physical signs occurring in hepatic cirrhosis which are occasionally of diagnostic value. It seems probable that they are of endocrine origin and that they are in the same category as spider nevi and other 'endocrine stigmata.'

Case Reports

Chronic Relapsing Pancreatitis with Associated Marked Eosinophilia and Pleural Effusion*

KERRISON JUNIPER, JR., M.D.

Boston, Massachusetts

MARKED eosinophilia associated with chronic relapsing pancreatitis is a rare phenomenon. The occurrence of a case in which eosinophilia and serum amylase were apparently correlated served as a stimulus to re-examine the records of sixteen other patients in which pancreatitis and eosinophilia occurred simultaneously. A review of the literature revealed no mention of eosinophilia occurring in association with pancreatitis.

CASE REPORT

First hospital admission: Patient W. F. H., a fifty-five year old white salesman, was admitted to another hospital on May 2, 1953, with a two year history of symptoms suggestive of congestive heart failure. There was also a history of epigastric pain with substernal radiation of several years' duration. Pain relationships were vague. All symptoms had become more intense for five months prior to admission. The immediate cause of admission was not clearly stated by the patient. There was a history of moderate alcohol intake. The patient had been hospitalized seven, nine and ten years previously for similar symptoms. On the last occasion he had been found to have hypertension and had received digitalis for a short time.

Examination revealed the patient to be afebrile and chronically ill, with a small left pleural effusion. Examination of the heart revealed it to be normal. The abdomen was protuberant with a fluid wave of questionable nature and with generalized tenderness, more marked in the left upper quadrant. The liver and spleen were not palpable.

Routine laboratory studies, liver function tests and stool examinations gave normal results. The initial impression was "possible cirrhosis of the liver and/or possible heart disease."

Four days after admission the patient became febrile and severe pleuritic pain developed in the lower left portion of the chest, with an increase in the left pleural effusion. Chest film revealed a density at the base of the left lung. At the same time symptoms of thrombophlebitis in the left leg appeared. A diagnosis of thrombophlebitis with pulmonary infarction was made. Serum amylase at this time was 220 units.† Signs of thrombophlebitis disappeared rapidly with treatment.

Eleven days after admission the abdomen became more distended and tympanitic, with tenderness localized to the left upper and left lower quadrants. The following day the tenderness was localized to the epigastrium and peristaltic sounds were absent. Signs of acute inflammation in the abdomen developed and laparotomy was performed thirteen days after the patient had been admitted. An inflammatory mass was found in the left upper quadrant of the abdomen extending down the left gutter to the level of the rectosigmoid junction, and cecostomy was performed. The liver appeared normal but adequate abdominal exploration was not possible because of dense adhesions and the patient's poor condition. Postoperative serum amylase was 800 units.

Mild abdominal pain and episodes of fever occurred postoperatively. On the eighteenth postoperative day the left pleural effusion had increased. Thoracentesis yielded 300 cc. of

† Amylase determinations were made by the modified Somogyi method[1] utilizing Nelson's method for determination of the blood glucose.[2] Normal values at this hospital range up to 200 units.

* From the Medical Service, V. A. Hospital, and Dept. of Medicine, Emory Univ. School of Medicine, Atlanta, Ga.

sterile, straw-colored fluid containing 800 units of amylase, 300 white blood cells and 40,000 red blood cells per ml. Two days later an additional 950 cc. of similar pleural fluid was removed, following which the fluid did not reaccumulate.

During the latter period of hospitalization, productive cough with some blood-streaking of the sputum, and night sweats. At this time he gave a five-year history of recurrent gnawing epigastric pain, and sharp pain aggravated by deep breathing in the left upper abdominal quadrant and left chest. Pain relationships were

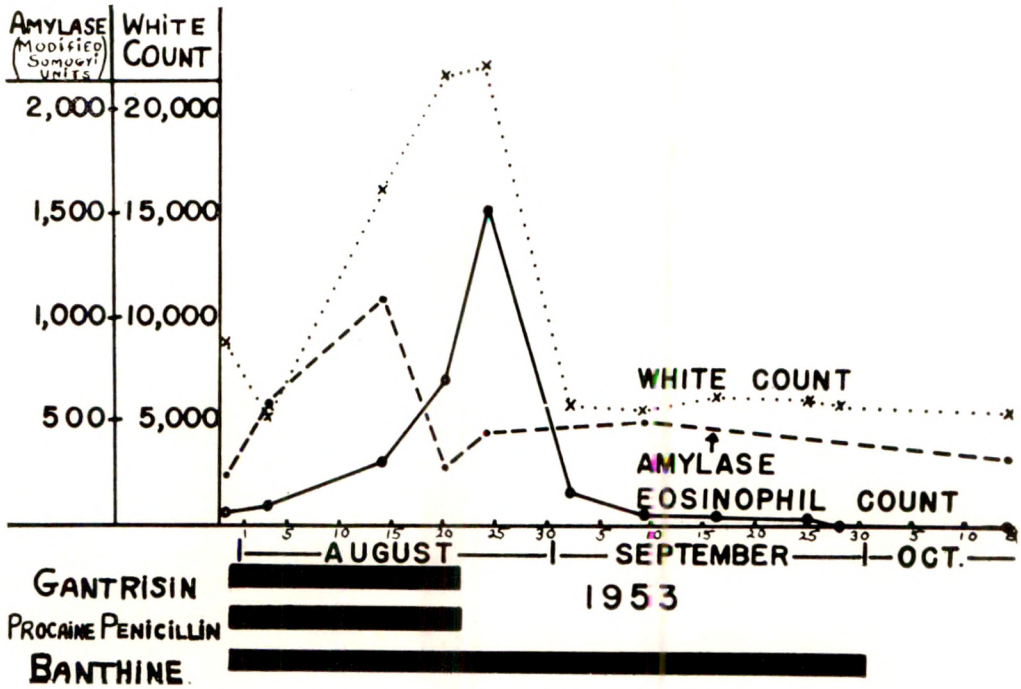


FIG. 1. Serum amylase and blood eosinophil levels during the second hospitalization period.

upper and lower gastrointestinal series gave normal results. Serial laboratory studies showed a white blood count of 5,300 with no eosinophilia on admission, and a rise of the eosinophil count to 550 in 11,000 white blood cells five days after admission.* Three days after surgery the white blood count was 15,000 with 750 eosinophils per ml. The patient was discharged two months after admission with a final diagnosis of acute pancreatitis.

Second hospital admission: The patient was admitted to this hospital on July 30, 1953, thirty-five days after discharge from his first hospitalization, complaining of dyspnea, chest pain and vomiting of several days' duration. During the preceding three weeks he had had a slightly poor, although alcohol had been noted to aggravate and milk to relieve the gnawing pain. He had had nausea but only occasional vomiting with these attacks of pain.

The patient was acutely ill, although afebrile, with moderate left-sided pleural effusion. The heart was normal. The abdomen was generally tender, especially in the left upper quadrant, where an ill defined mass was palpated. The liver and spleen were not palpable.

Amylase levels, white blood cell, and eosinophil counts are shown in Figure 1. Of particular note are the maximum white blood cell count of 22,150 taken during the fourth week of hospitalization and the marked eosinophil counts of 11,935 and 15,284 per ml. during the third and fourth weeks of hospitalization. Laboratory tests for tuberculosis and intestinal parasites gave negative results. Several serologic tests for syphilis gave positive results on admission but reverted to normal after the acute illness had subsided. Bromsulphalein retention

* Total eosinophil counts in this paper were estimated by multiplying the total white blood cell count by the percentage of eosinophils found on the routine differential count. In a few instances direct total counts were done to verify the degree of eosinophilia found. An eosinophil count of 200 per ml. was considered the upper limit of normal.

was 20 per cent in one-half hour (5 mg. dye per kg. of weight), serum bilirubin 2.1 mg. per cent, and serum proteins 7.2 gm. per cent with 3.9 gm. of albumin and 3.3 gm. of globulin. Flocculation tests of liver function gave normal results. Serum amylase was 237 units. Electrocardiogram was normal.

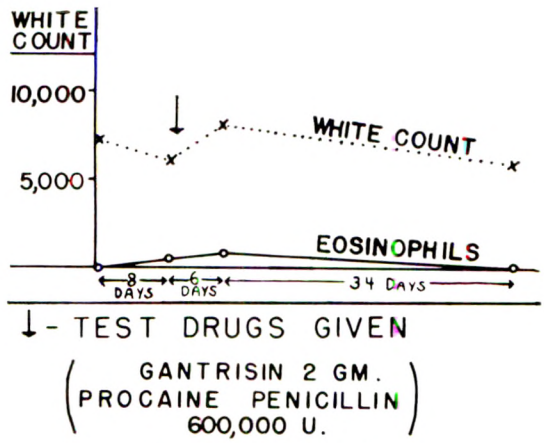


FIG. 2. Sensitivity study.

On admission 400 cc. of thin, sterile, straw-colored fluid were removed from the left pleural cavity. This fluid contained 5.8 gm. per cent protein, 2,375 white blood cells and 5,000 red blood cells per ml. Differential count of the pleural fluid cells showed 2 per cent polymorphonuclears, 8 per cent lymphocytes, 1 per cent eosinophils, 1 per cent basophils, 85 per cent histiocytes and 3 per cent mesothelial cells.

During the first week of hospitalization the patient was febrile with temperatures as high as 101°F. He received procaine penicillin and gantrisin® for the first twenty-one days, and banthine® during the entire period of hospitalization. During the last five weeks there was gradual improvement with resolution of the pleural effusion.

One month after admission a repeat bromsulphalein test gave normal results. Upper and lower gastrointestinal and gallbladder series gave normal results. Serial chest films showed gradual resolution of the pleural effusion with no evidence of a parenchymal lesion. The highest serum amylase level occurred two weeks after admission and was 1,094 units. The patient was discharged ten weeks after admission with a diagnosis of chronic relapsing pancreatitis.

The patient has been followed as an outpatient for a period of one year, during which time he has had approximately one attack per month of abdominal pain with nausea and vomiting, each attack lasting about five days. Follow-up chest films have shown no return of the pleural effusion, and serum amylase levels have remained within normal limits. A very slight eosinophilia has been noticed occasionally.

In order to investigate the possibility of a drug sensitivity, the patient was studied during a relatively asymptomatic period six months after his discharge from the hospital. Unfortunately, patient cooperation was poor and laboratory tests could not be done at the desired times. Control eosinophil counts were made and the patient given 600,000 units of procaine penicillin intramuscularly in a single dose and 4 gm. of gantrisin orally over a forty-eight-hour period. Figure 2 demonstrates the results of the sensitivity test. At no time during the test did any symptoms or signs of an allergic reaction develop. It was unfortunate that the second control eosinophil count had risen to an abnormally high value (600 per ml.). The additional rise of only 200 eosinophils after drug administration seemed insignificant in comparison with the 527 rise which occurred between the control counts. The total eosinophil rise during the test seemed particularly insignificant in relation to the maximum eosinophil count of 15,284 per ml. which appeared during the acute illness. The conclusion drawn from this test was that a drug reaction to either procaine penicillin or gantrisin was an unlikely cause of the eosinophilia which occurred in association with the acute attack of pancreatitis.

REVIEW OF SIXTEEN CASES OF PANCREATITIS WITH EOSINOPHILIA

The relationships found in the case presented suggested a review of other cases of pancreatitis. The serum amylase determinations done at this hospital during the previous nineteen months were examined and the records revealed that sixty patients had had amylase values of 200 or more units. Of these forty-two also had total eosinophil counts of over 250 per ml. There was adequate clinical data on sixteen of this group with eosinophilia to substantiate a definitive diagnosis of pancreatitis of either the acute or chronic type. Eight patients had eosinophil counts of over 500 per ml., and those of two patients were above 1,000 per ml. In no case, however, was there any obvious relationship between the activity of the disease and eosinophilia, although a mild-to-marked eosinophilia

was present. There were no other obvious causes of the eosinophilia in this group. The type of study done and the small number of cases found did not permit evaluation of the significance of eosinophilia generally in pancreatitis.

COMMENTS

The eosinophilia which appeared in this patient during the second period of hospitalization was very striking. Intestinal parasites, first thought to be the cause, could not be found. The course of the disease made periarteritis, trichinosis and malignancy (other common causes of marked eosinophilia) seem unlikely. The possibility of a drug reaction was considered because the patient had received procaine penicillin and gantrisin during the time he had eosinophilia. The drug sensitivity test, however, made this seem unlikely.

Plotting the serum amylase values and total eosinophil counts (Fig. 1) revealed an interesting relationship. It was noted that ten days after the peak amylase value (representing in this case the more acute period of the disease process) the maximum eosinophil response appeared. This lag resembled the ten to fourteen-day period frequently seen before the delayed systemic response manifests itself after exposure to an allergen. This is not an indication that the eosinophilia in this patient was the result of an allergen (such as altered pancreatic proteins) released during the acute inflammatory process, although it remains a possibility. Whatever the cause, it was the impression of those associated with this case that the eosinophilia was directly related to the acute pancreatitis. A third severe attack of pancreatitis has not occurred to date in this patient and consequently further observations on eosinophilia and acute pancreatitis have not been possible.

This case also demonstrates the difficulty frequently encountered in the initial diagnosis of acute pancreatitis. That pleural and peritoneal effusions occur in conjunction with acute pancreatitis is well known. Less well known is the fact that such effusions may contain increased amounts of amylase and that such levels may be higher than blood serum values and remain elevated longer. The presence of pleural effusion containing 800 units of amylase in this case was of particular interest. Various mechanisms have been suggested as the cause, varying from inflammation of the diaphragmatic pleura by adjacent abdominal inflammation or a subdiaphragmatic abscess, to lymph drainage of high enzyme content from the abdomen through the diaphragm into the pleural cavity. The presence of large amounts of amylase in the pleural fluid makes the latter theory seem plausible, although it is possible that diffusion from the blood serum also may occur. Reports that the serum-amylase levels have been found to be lower than the pleural fluid content at the same time make this less likely. However, there has not been adequate investigation to rule out the possibility that the pleural fluid amylase content diffused from the blood serum at an earlier time and merely persisted after the blood amylase level had fallen.[3–9]

SUMMARY

A case of chronic relapsing pancreatitis with pleural effusion on the left side is presented. During an acute exacerbation marked eosinophilia was observed which appeared to be related to the activity of the inflammatory process. Eosinophilia of a mild-to-marked degree was found in sixteen other cases of definite pancreatitis but no obvious relationship between eosinophilia and degree of activity (including amylase values) was found. No mention of a similar relationship between eosinophilia and pancreatitis was found in the literature.

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Aneurysm of the Left Coronary Artery*

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ONLY fifty cases of localized aneurysm of the coronary artery have been reported in the literature. Bougon in 1912 was the first to describe such a finding. Packard and Wechsler[1] in 1929 reported a total of twenty-nine cases, including one of their own. They classified these cases according to an arteriosclerotic or mycotic-embolic etiology. Scott[2] reported another case in 1948 and in a review of the literature compiled forty-seven cases. Since Scott's paper appeared only single case reports by Sarkisian[3] in 1950, Rukstinat[4] in 1952 and Colbeck and Shaw[5] in 1954 have been reported, thus bringing the total number of reported localized coronary aneurysms to fifty.

CASE REPORT

A sixty-nine year old white woman, markedly obese, was admitted on June 1, 1954, complaining of progressive severe substernal pain. Marked dyspnea and ankle edema were present. This admission was the last of many beginning in 1942 for hypertension, congestive failure and symptoms of coronary insufficiency. No history of rheumatic fever, syphilis, subacute bacterial endocarditis or myocardial infarction was present. On this last admission the patient's blood pressure was 130/70 and her pulse was 75. Her usual pressure was about 200/100. She was markedly dyspneic, pale and sweaty. Fine moist rales were heard bilaterally in both lung bases and the heart sounds were of regular rhythm and barely audible. The abdomen was very obese; no viscera or masses were definitely palpable. A +2 pitting edema of the ankles and feet was noted. An x-ray of the chest showed left ventricular enlargement and a small amount of fluid at the left base. A review of previous electrocardiograms revealed progressive coronary insufficiency. An electrocardiogram taken during the last twenty-four hours of the patient's life showed anoxia of the anterior wall of the left ventricle and auricular fibrillation. These observations were not present in previous electrocardiograms. The course in the hospital was marked on one occasion by a period of unconsciousness and transient left hemiplegia which cleared in one and one-half hours. Recurrent bouts of chest pain with left shoulder radiation also took place. Three days before death the patient became febrile and suffered severe nausea and vomiting. Severe pulmonary edema developed on June 30 and the patient expired.

At autopsy, marked bilateral pulmonary edema was present. The heart weighed 500 gm.; all chambers were moderately dilated. The left ventricular wall measured 16 mm. and the right ventricular wall measured 8 mm. The mitral and aortic valves showed moderate sclerosis at the free edges but did not show evidence of rheumatic heart disease or subacute bacterial endocarditis. The entire aorta showed moderate involvement by atherosclerosis but no evidence of syphilis was noted. On sectioning the cardiac muscle focal streaky areas of fibrosis were revealed. Both coronary ostia were adequately patent. The coronary arteries presented a balanced distribution with slight focal atherosclerotic involvement.

Two centimeters distal to the ostium of the left coronary artery a small coronary branch 1 mm. in diameter was observed which coursed downward to the right. This branch, 1½ cm. from its origin, led to a saccular aneurysm measuring 5 cm. in diameter which reached to, overlaid and apparently compressed the main anterior descending branch of the left coronary artery. The aneurysm contained an organized peripheral laminated blood clot with central fresh hemorrhagic material. The wall of the aneurysm measuring 1 mm., was markedly thin and showed focal points of ulceration and

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calcification. From its position the aneurysm could have pressed down upon and thus have been responsible for intermittent occlusion of the anterior descending coronary artery. (Fig. 1.)

Microscopic sections of the aneurysm wall revealed destruction of the inner elastic membrane. Necrotic atheromatous material and foci of calcification were noted on the inner aspect of the wall. One area revealed a site of definite bone formation. The media was markedly fibrosed in many areas. From these histologic features it is apparent that the aneurysm was not of recent origin.

Histologic sections of the major coronary vessels revealed only minimal atherosclerosis. Several compressed coronary branches were noted in the vicinity of the aneurysm. The heart muscle itself showed numerous disseminated areas of focal fibrosis and moderate fiber hypertrophy. (Fig. 2.)

The final diagnoses of the pathologist were congestive failure secondary to hypertensive arteriosclerotic cardiovascular disease, and an arteriosclerotic aneurysm of the main left coronary artery.

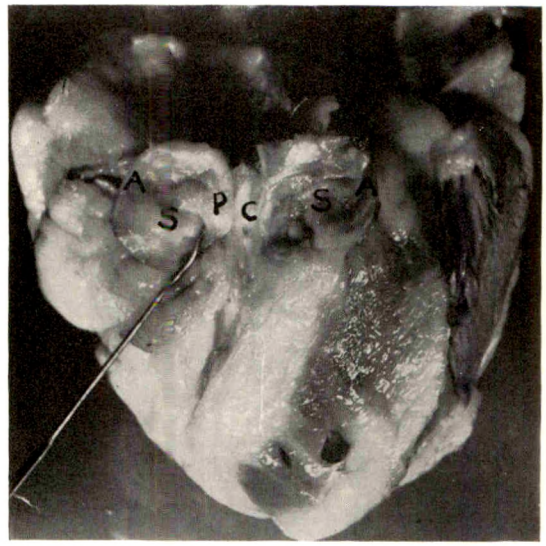


FIG. 1. Aneurysm incised to show its straddling position over the anterior descending coronary artery. A, wall of aneurysm; C, anterior descending coronary artery; P, probe in lumen of small coronary branch; S, sac of aneurysm.

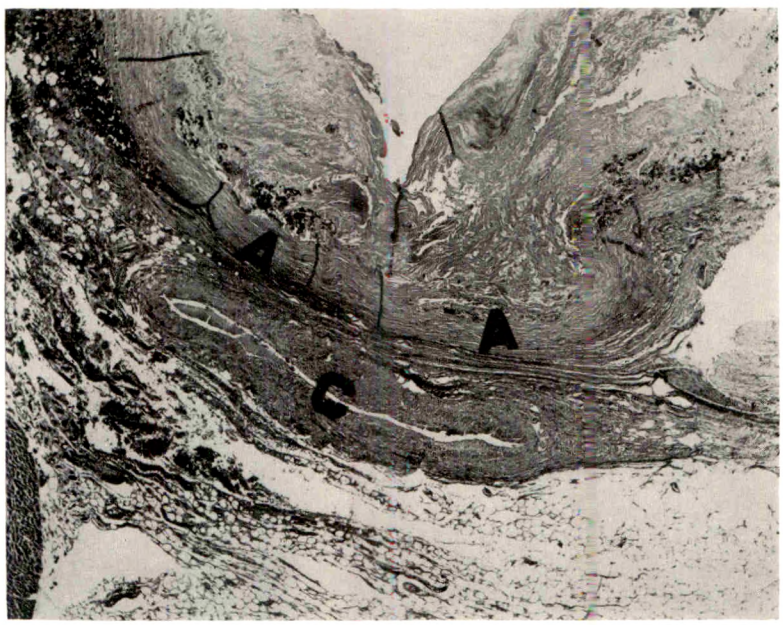


FIG. 2. Wall of aneurysm overlying and compressing coronary artery branch. A, wall of aneurysm; C, compressed coronary artery branch.

COMMENTS

Scott classified the aneurysms in his compilation as localized and diffuse. The localized aneurysms were further subclassified etiologically as congenital, fifteen patients; mycotic-embolic, twelve; arteriosclerotic, six; syphilitic, six; purely mycotic, one; rheumatic, one; possible periarteritis nodosa, two; and unclassified, four. The left coronary artery was involved in twenty-seven patients, the right coronary artery in eleven, and both were involved in six pa-

tients. In three patients the sites of involvement were unstated. The commonest causes of death in the localized aneurysm were coronary thrombosis, congestive heart failure and coronary rupture.

In reviewing the literature it is found that a single aneurysm of the coronary is usually located immediately distal to the coronary orifice within the first inch of the origin of the vessel. In mycotic-embolic aneurysms the sac usually contains infected embolic material. The infection first involves the intima and spreads through the media and adventitia. Leukocytic infiltration and destruction of the internal elastic membrane ensue with secondary destruction of the media. Pure mycotic aneurysms are usually associated with septicemia. Aneurysms due to arteriosclerosis show destruction of the internal elastic lamina at the site of the atheromatous plaque along with destruction and atrophy of the media. The three single case reports since Scott's paper all occurred against an arteriosclerotic background. It is interesting to note in our case report that although the aneurysm was also on an arteriosclerotic basis, it was associated with minimal arteriosclerotic involvement of the remainder of the major coronary branches. This observation has been previously noted by Scott. It is possible therefore that a factor contributing to the progressively severe angina and cardiac failure experienced by our patient during her last year of life may have been external pressure of the aneurysm on the left anterior descending coronary branch. This view is further supported by the widespread myofibrosis and lack of any occluding atherosclerosis elsewhere.

SUMMARY

A case of localized aneurysm of the left coronary artery is presented. This is believed to be the fifty-first case reported in the literature. Ten of these aneurysms, including the one herein reported, were on an arteriosclerotic basis.

Death from aneurysm is usually due to congestive failure, coronary thrombosis or rupture. In our study it is postulated that intermittent occlusion of the left anterior descending coronary artery by external pressure from the overlying aneurysm may have contributed to the terminal manifestations of congestive heart failure.

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The American Journal of Medicine

VOL. XIX NOVEMBER, 1955 NO. 5

Foreword

THIS conference on myasthenia gravis sponsored by the Myasthenia Gravis Foundation was held at the Medical School of the University of Pennsylvania on December 8 and 9, 1954. Presiding at the various sessions were Dr. Henry Viets, Chairman of the Medical Board of the Foundation, Dr. Elmer Severinghaus and Dr. George Morris Piersol, Dean of the Graduate School of the University of Pennsylvania.

The objectives of the conference were conceived as a review of current investigations on the disease and its therapy, to summarize present concepts of the physiology of neuromuscular activity and to discuss directions of future study. In several papers the influence of endocrine factors on neuromuscular function in normal states and in this disease was examined. Dr. Venning in her review on the endocrine changes in pregnancy considers whether or not such alterations might bear on remissions of the disease sometimes occurring in pregnancy.

The papers are divided into sections dealing with the various aspects of this subject. The first includes a statement of the clinical problem by Dr. Viets and a review of normal neuromuscular transmission; the others discuss the pharmacology of neuromuscular transmission, the pathologic physiology of myasthenia, the thymus and myasthenia, the endocrine glands and myasthenia, and the drug therapy and management of the disease.

This introduction attempts to call attention to some of the chief points brought out in the discussion. All except two of the original papers are published here. Space limitations unfortunately precluded the use of most of the discussion except in three instances where factual information of importance was added.

The Foundation wishes to acknowledge the cooperation and generosity of Mr. Elmer Bobst, Ciba Pharmaceutical Products, Hoffmann-La Roche, Inc., Smith, Kline and French Laboratories and Winthrop-Stearns, Inc.

DISCUSSION OF PAPERS

The modern era in the study of this disease began about 1935 with the neurohumoral theory of Loewi and Dale and the realization that the disease, which resembled curare poisoning, could be reversed by the antidote, eserine, and later by prostigmin. These observations led to three lines of study: (1) A comparison was made, by electromyographic means, of the disease with the normal process and with the blocking effect of curare and other agents. (2) Because of the analogy to curare poisoning, efforts were made to discover a circulating toxin, especially directed toward the thymus gland as a source. (3) A study of therapy with new drugs having anticurare or anticholinesterase activity was done.

Meantime, our knowledge of the process of normal nerve-muscle activity has been elaborated, and Drs. Nastuk, Koelle and Nachmansohn* reported some of the current developments in this field. The nature of the action of blocking agents was reviewed for us by Dr. Van Maanen and Dr. Churchill-Davidson and discussed by others. The myasthenic disability was compared to the normal process and to the action of these blocking agents by Drs. Johns, Grob and Harvey, Dr. Churchill-Davidson and Dr. Botelho.

Concerning the normal process, we have learned that acetylcholine is held in a bound form and released upon stimulation, whereby it effects a contraction of muscle. It is promptly broken down by the enzyme cholinesterase into choline and acetate and again resynthesized by another enzyme, choline-acetylase, into the bound form. The action of acetylcholine is to depolarize the membrane, which is accompanied

*Paper not included in this issue.

by shift of sodium into the cell and potassium out of the cell. Nastuk pointed out that this process is influenced by the composition of the extracellular fluid, being greatly impeded by withdrawal of sodium. He considers that acetylcholine does not act by shifting into the endplate because acetylcholine liberation is not influenced by a change in extracellular sodium, although the depolarization process is. Dr. Nachmansohn discussed the action of quaternary nitrogen compounds in terms of their binding properties by physiochemical action to the receptor proteins which he distinguishes from the specific enzyme proteins previously mentioned. In their relative binding he sees an explanation for the lesser effect of tertiary nitrogen compounds compared to the quaternary group. Dr. Koelle showed by histochemical means that the specific enzyme cholinesterase concentrates about the palisade-like subneural apparatus of the endplate. He also reported that this enzyme has now also been found in adrenergic nerves and posterior roots in small concentration, as had previously been known of the cholinergic nerves. He suggested that the endplate region should be studied by these methods and compared to the normal.

As a result of studies of blocking agents, two types have been described, namely, a depolarizing block and a non-depolarizing block. The studies of Drs. Johns, Grob and Harvey suggested that a continuing block exists in the resting myasthenic muscle, since its action potential is below normal. They consider this to act as a competitive type of block due to some substance liberated as part of the normal process, probably choline. They present evidence showing that the myasthenic abnormality can be duplicated by administration of choline. This seems to be an important advance in our concept of the nature of the defect in myasthenic muscle at its endplate. Presumably the view formerly held by these investigators that lessened sensitivity of the myasthenic endplate to acetylcholine constituted the major change in this disease has given way to this new concept.

Dr. Churchill-Davidson presented evidence showing that the action of decamethonium in the myasthenic differed from that in the normal subject. In the latter a depolarizing block occurs but in the former a combined depolarizing and non-depolarizing block develops. Unaffected muscle in myasthenics revealed resistance to depolarization. Dr. Grob raised the question as to whether or not a faster rate of stimulation than that employed might have given different results. I assume Dr. Churchill-Davidson accepts this point.

Two papers drew attention to the muscle itself in contrast to endplate blocking agents. Dr. Botelho reported that the tension curve is a better index of the weakness than the electromyograph which may not be affected in the same direction as the tension curve. Whether or not this is a defect of the myograph technic or a fault of the disease is open to question. Dr. Shy* reported his early studies of sodium and potassium concentration in intra- and extracellular water in human biopsy specimens. These have not yet shown any consistent pattern, but have not confirmed Cummings' findings of the accumulation of potassium in myasthenic muscle.

The theory that the thymus gland may contain and release a blocking or depressing substance in myasthenia gravis has been the object of study by Professor Andrew Wilson for several years. In summarizing his latest findings he noted that extracts of the thymus glands, especially from the fetal whale, contained a depressing substance. The action of these extracts does not resemble that of the ordinary blocking drugs and is not influenced by prostigmin. The nature of this material is unknown. Thymic extracts made by another technic, as pointed out by Dr. Zack, may contain potassium in amounts sufficient to account for some of the depression observed. The efforts of Dr. Rider to show a difference in the effect of thymic extracts from myasthenic and normal subjects, or of serum from myasthenic subjects failed to demonstrate any difference in cat neuromuscular preparations. Dr. Schwartz's animals on injection of thymus extract showed either convulsions or spasm along with vomiting, collapse and weakness. It would seem therefore that at the moment we are unable directly to relate the thymus, on the basis of this type of experimental work, to the production in animals of a condition resembling myasthenia gravis. Such a relationship still remains to be demonstrated. Dr. Van Maanan pointed out that the method of assay for blockading agents used in some of these studies may be too insensitive, for it does not show up curare in the serum of an animal which is fully paralyzed by the drug.

On the other hand, the evidence of thymic relationship to myasthenia gravis seems to be accumulating. Dr. Eaton's analysis appeared to

* Paper not included in this issue.

confirm the observations of the Boston group that thymectomy in young women exerts a favorable influence as compared with those in control groups. What this will ultimately mean in terms of therapy and understanding of the disease remains to be seen. Sceptics like Grob are still unwilling to concede that thymectomy is any more than a continuing experiment. However, the evidence as it accumulates seems to favor the point of view that thymectomy may influence the disease.

The next section constituted an examination of endocrine factors other than thymus in this disease. These papers were listed to review the effect of endocrine changes in pregnancy and in work, as well as the influence of endocrine diseases and administration of hormones upon myasthenia gravis. In pregnant women who have this disease an important observation is the fact that certain of the babies of myasthenic subjects have myasthenia gravis (presumably transmitted from the mother) which clears up in a short time. As to the effect of pregnancy on the disease in the mother, the data presented by Drs. Schlesinger, Osserman and others shows that a third of the patients became worse, a third remained unchanged and a third improved. Although these figures seem to provide no direct relationship, no doubt some subjects improve remarkably during pregnancy, usually in the latter part, and relapse after delivery. For these patients one does not like to abandon the possibility that there might be an association.

Dr. Ingle discussed the endocrine compounds (emphasizing the adrenal cortical extracts) which favorably influence the work performance of the normal rat and described the favorable action of posterior pituitary hormones. Dr. Kane gave an excellent review of the harried subject of endocrinopathies in relation to the myasthenic process. In this paper opinion seemed to crystallize that ACTH has an adverse effect upon this disease, despite contrary views previously published. Although he emphasized that improvement in myasthenia occurs from thyroid therapy more often than the reverse, many have felt that hyperthyroidism unfavorably influences the disease. The association here again is puzzling in that it is not direct.

In the section on drug therapy two new compounds were discussed. The advantages and the disadvantages of their longer action and of the lesser parasympathetic effect were noted. The so-called cholinergic crisis produced by these and other drugs is a very real problem. Many persons have thrown themselves into this serious condition by inadequately supervised self-medication.

As Dr. Viets has stated, the mortality rate of myasthenia has been reduced to about 10 to 15 per cent by modern therapy, yet as far as we know these drugs are symptomatic therapy and do not influence the disease process directly. They are not in any way concerned with development of remissions. To me it seems that the therapy of the future should be concerned with the possibility of inducing a remission rather than treating the established disability. Fatalities continue to occur, and a more definitive method of treating this disease is needed. It must be pointed out that should we discover, as a cause of the defect in myasthenia, a circulating toxin, our problem may not thereby be solved. One needs only to recall that the cause of botulism is known, yet no therapy is effective. The same might be said of tetanus. Both of these conditions have a resemblance, although in opposite directions, to the myasthenic process.

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Symposium on Myasthenia Gravis

Problems of Myasthenia Gravis

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THE problems surrounding myasthenia gravis are multiple and diverse. Many questions posed by the disease cannot be answered with any degree of confidence at the present time. Some, particularly those of a clinical nature, have been resolved by the discriminating observations of patients by previous workers, for the disease has been adequately recognized since Erb's description in 1879 and Goldflam's in 1893; Jolly's nomenclature was suggested in 1895 and generally accepted after 1899. Most of the clinical facts were known by 1900 and Weigert, a year later, had even disclosed a thymoma in one of Laquer's patients at autopsy.

Willis had of course grasped the one essential sign as early as 1672, namely, the faulty and impaired weakness of voluntary muscles evoked only by repeated stimulation with a tendency toward recovery upon rest. Thus the disease having only one sign was relatively easily diagnosed by our predecessors, even as it is today in most cases. By 1900 much of the symptomatology was recognized: age, sex, sudden onset after emotional or physical trauma, and variations at puberty and during the puerperal state. Localization with common muscular groupings relative to cranial nerve innervation, neck weakness, and weakness of extremities and back, had been fully described. From 1900 to 1934 the disease was considered uncommon and usually fatal. No pathologic changes were noted in the nervous system and only the thymic abnormality and "lymphorrhages" in the skeletal muscles were noted. The stimuli which led to our present day concept for myasthenia gravis came largely through the discovery of the junctional transmission of nervous effects by chemical agents by Otto Loewi (1921), Henry H. Dale (1933) and others, the striking response of physostigmine and later neostigmine in overcoming the muscular deficiency in patients by Mary B. Walker (1934) and the suggested relationship between the thymus and myasthenia gravis by Blalock, Keynes, Andrew Wilson and others (1941 to date). These and other contemporary investigations have revived an interest in the disease (which had nearly died out after the turn of the century) and led to the establishment of a Myasthenia Gravis Foundation. The calling of this Conference in 1954 was for the purpose of exchanging ideas on the problems presented by twenty years of research since Walker's brief letter to the editor of the *Lancet* was published on June 2, 1934. Few physicians in the history of medicine have been more privileged than Mary Walker in establishing a new trend in medicine, accomplished by acute clinical observation at St. Alfege's Hospital in the Spring of 1934. This Congress and indeed the whole myasthenia gravis awakening owes a debt to her; by meeting here today we are attempting to repay a debt, long overdue.

I would like to take this opportunity to discuss briefly three main problems, each presenting fundamental questions of importance to our understanding of myasthenia gravis.

THE PROBLEM OF JUNCTIONAL TRANSMISSION

Prior to Loewi's classical demonstration of the release by vagus and sympathetic impulses of substances resembling acetylcholine and adrenaline, T. R. Elliott had made such a suggestion as early as 1904. It was not until 1929, however, that Dudley and Dale were able to recognize acetylcholine as a natural body constituent and later suggest that peripheral nerve impulses liberated this substance at the synapse. Although no one has been able to collect a sufficient quantity of acetylcholine transmitting the effects of nerve impulses at the endplate area to be able to identify it chemically, acetylcholine is a very unstable choline ester, rapidly destroyed by cholinesterase and synthesized by cholineacetylase. However, the transmitter may be concentrated at the nerve ending together with the

specific enzymes for its formation and its destruction; the chemical may also be found along the nerve fiber and perhaps even at the cell of origin. The system is not specific for the nerve ending alone. It cannot be assumed that acetylcholine must act as a stimulator for all cells or fibers from which it can be extracted. The problem needs further elucidation, but in myasthenia gravis at least it would seem that the muscle at its receptive area in contact with a cholinergic fiber end is sensitive to acetylcholine or, as Dale put it, "cholinoceptive." It is possible that cholinergic function and cholinoceptive sensitiveness are different characters which need not, and often do not, go together.

We need to know where the acetylcholine and its enzymes originate. Do they come from the nerve cell and pass down the fiber to maintain a physiologically effective depot at the ending? These and many other problems await the answers from neurophysiologists, the final interpretation of which may profoundly affect our concept of what happens in myasthenia gravis when the cholinesterase is inhibited by neostigmine and the system, at least for the time being, is set right and function is restored. We are far from our goal. Can we be certain that all nerve impulses are released by chemical transmitters and, if they are, can all the transmitters be identified? We need further information regarding one-way restriction, the effects of poisons and the part played by many drugs influencing this system of nerve transmission. Much is in the realm of pharmacology, aided by the electrical and biophysical methods of physiology. To these tasks a research program for the Myasthenia Gravis Foundation should in part be devoted. Although the chance clinical observation of Mary Walker has resulted in prolonging many lives and making possible a wider study of the disease and its course in man, we can not hope for many inspirational flashes of a similar nature for, welcome as they are, medical advance fundamentally depends on well planned experimentation without limit of time or substance.

THE THYMUS AND MYASTHENIA GRAVIS

That the thymus bears some relationship to myasthenia gravis is now widely accepted. What the relationship is leads us at once into a field of physiologic theory and clinical speculation. The evidence for thymic involvement rests on the pathologic findings of thymic change in a large percentage of patients with myasthenia, the favorable results of thymectomy in selected cases of the disease, the formation of thymomas in about 15 per cent of cases with thymic abnormalities and the experimental evidence of Wilson which revealed that an extract of the thymic tissue removed by thymectomy appears to depress contractions in muscle-nerve preparations to a degree comparable to that produced by tubocurarine. Thus writes Keynes on the "physiology of the thymus gland," and Levitt went so far as to refer to "thymic myasthenia." Many would feel, with considerable justification, that conclusions of this type have not been established to the point of acceptance. There is still need for continued investigation, and profit may be expected to result from extended studies of postoperative patients, particularly in selecting those most likely to be improved by thymectomy and in evaluations of experimental extracts along the lines suggested by Wilson and others. Does the thymus play a substantial or even a subsidiary function in myasthenia gravis? Is it subordinate to the pituitary or others in the endocrinological system? One can hardly conceive of such a prominent structure, so profoundly affected in myasthenia gravis, as having no relationship to the disease. Thymic studies are of primary importance in expanding our knowledge of the disease. Such a promising field should not be neglected.

THE PROBLEM OF MEDICAL CARE

Shortly after 1934 neostigmine (prostigmin) became the standard form of treatment for patients with myasthenia gravis. In 1954 it still holds a primary place in overcoming the muscular deficiency for it has shown no tendency toward habit formation, it rarely becomes ineffective because of tolerance and has maintained its effectiveness throughout the years; it is relatively easily adjusted to suit the patient's needs and its muscarine-like stimulation of the parasympathetic nerves is usually readily controlled by atropine. On the other hand, the drug has the defect of being short-acting, the usual response from oral medication being one to three hours and, when given intramuscularly or intravenously, much shorter. Thus many patients require some medication every two hours during the day and every three or four hours during the night. Many drugs have been given a trial during the last twenty years, including other anticholinesterase preparations, but

none have been found as useful over a long period of time as neostigmine. As the use of the drug in myasthenia gravis is virtually one of replacement therapy, only that medication will be satisfactory that can be taken with safety and results in a minimum discomfort from overdosage or underdosage. Recently pyridostigmine (mestinon®) has shown a tendency to replace neostigmine, but the search for a better and longer lasting drug must continue, and this Conference may stimulate research along these lines. Our knowledge of neostigmine is still lacking for we do not know where it is absorbed in the gastrointestinal tract, how it is eliminated, where it is stored in the body and what blood level is obtained. It is not known when or why some patients with myasthenia gravis can apparently use to advantage 1,500 mg. or more of the drug in a day, whereas 15 or 30 mg. is not easily tolerated by normal persons. These and other similar problems call for answers before our therapy can be improved and before we can reach a goal of full and continued maintenance of muscular power.

In posing three major problems, many others will occur to all of us. The basic cause of the disease still eludes us, as do the reasons for its onset at various age levels, the cause of relapse and remission progression, the transmission of the symptoms to the child by the pregnant mother with the disease, the reasons for its variable location in particular muscle groups and, finally, the problem of atypical cases or those showing poor neostigmine response need further elucidation. Such problems set a pattern for future research.

Structure of the Motor Endplate*

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In his comprehensive review on the innervation of skeletal muscle, Tiegs[1] quotes the statement made by Kuffler[2] that "the end plate has become, in recent years, a physiological entity which at present cannot be strictly identified with the structure of the histologists." One reason for this hiatus in our knowledge is the disagreement which exists among histologists concerning the embryologic derivation and the limiting boundaries of some of the structures which comprise the endplate. The controversial points are discussed in detail in Tiegs' review; the present description is drawn chiefly from the pioneer work of Kühne[3] and the more recent studies of Couteaux[4,5] and Young[6] and their associates.

Centrally to its point of contact with the muscle fiber, the motor axon is ensheathed in a layer of myelin which is closely invested by the Schwann cells. The latter are surrounded by a continuous sheath called the neurilemma external to which is the sheath of Henle, a continuation of the perineural sheath surrounding the entire bundle of nerve fibers. Near the endplate region the myelin sheath is lost, and the sheath of Henle becomes continuous with the outer sarcolemma, an extraneous sheath derived from the endomysium. According to Couteaux,[4] at the endplate the axon retains a surrounding sheath, the teloglia, derived from the sheath of Schwann, and remains external to the true sarcolemma. The latter is modified, beneath the axon with its investing teloglia, as the pallisade-like subneural apparatus. Whether the neurilemma or a derivative is retained is less certain. Surrounding the subneural apparatus is a fairly well circumscribed region of granular sarcoplasm which is rich in nuclei. The entire motor endplate occupies a third or less of the circumference of the muscle fiber.

Couteaux has focussed attention on the subneural apparatus as the probable site of both the acetylcholine(ACh)-receptor substance, where the endplate potential is initiated, and most of the acetylcholinesterase (AChE) of the endplate. The subneural apparatus was demonstrated initially by selective staining with Janus Green B, and was found to have a lamella-like structure. The same pattern is obtained when the endplate is stained for AChE[5] by the acetylthiocholine method,[7,8] whereas staining for the enzyme is considerably lighter in the axon and its terminal arborization. Thus in accordance with earlier deductions[9,10] most of the AChE at the endplate is postsynaptic and proportionately little is presynaptic. This is the reverse of the situation present in the majority of the synapses in sympathetic ganglions[8,11] and may account for some of the physiologic and pharmacologic differences between neuromuscular and ganglionic transmission.

Couteaux's depiction of the interposition of the teloglial sheath between the axon and the subneural apparatus is analogous to the situation in autonomic ganglia described by de Castro.[12] According to the latter author the preganglionic fibers are ensheathed in glial cells and thus do not make direct contact with the ganglionic neurons or their dendrites. If both conceptions are correct the influence of the glial elements on ganglionic and neuromuscular transmission under normal and pathologic conditions must be considered. The ganglionic glial cells and the Schwann sheath cells, from which the former take their origin, contain non-specific or pseudo-ChE, the function of which remains to be determined. The suggestion that the enzyme is responsible for maintenance of the myelin sheath[13,14] has been disputed.[15] It is quite possible that the low concentration of non-specific ChE recently demonstrated by Denz[16] at the motor endplate is localized in the Schwann cell–derived teloglia.

Until recently, histochemical findings indi-

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cated that the AChE of the nervous system was present only in cholinergic neurons, including those giving rise to motor fibers, preganglionic fibers and cholinergic postganglionic fibers, and certain neurons of the central nervous system. By the use of a selective inhibitor* of AChE to rule out staining due to hydrolysis of the histochemical substrate (acetylthiocholine) by other enzymes, and by employing a prolonged incubation period (two hours), it has now been found that adrenergic and sensory neurons contain relatively low concentrations of AChE.[17] This requires modification of the previously expressed conclusion[8] that histochemical evidence is wholly incompatible with Nachmansohn's[18] theory of the role of acetylcholine in the conduction of nerve impulses, the general aspects of which have been discussed by Eccles,[19] Gerard[20] and Bullock.[21] The significance of the small amounts of the enzyme present in adrenergic and sensory neurons, the acetylcholine and choline acetylase concentrations of which are also extremely low, has not been established.

Although there have been reports of nonspecific pathologic changes in the muscle fibers of patients with myasthenia gravis,[22] there is practically no information available concerning possible alterations in the details of the structures described. Studies employing specialized histologic and histochemic technics should be undertaken, in conjunction with physiologic and biochemic investigations, in future attempts to elucidate the nature of this disease and thereby advance the therapy.

* 1-5-bis(4-allyl dimethylammonium phenyl) pentan-3-one dibromide or B.W. 284c51.

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Neuromuscular Transmission*

Fundamental Aspects of the Normal Process

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In the vertebrate animal the normal neuromuscular transmission process involves the following sequence of events: nerve action potential → acetylcholine release → combination of acetylcholine with endplate membrane receptor → decrease in transmembrane potential at the endplate → muscle action potential → muscle contraction. These steps are considered from the biophysical point of view in two recent reviews.[1,2] In this article discussion is limited to a few selected topics chosen to illustrate certain principles and to point out some modern research technics now used in the study of neuromuscular transmission.

Initiation of Contraction; Muscle Cell Membrane Potentials. We may begin with the last step of the transmission process, namely, shortening of the contractile system in the intact muscle cell subsequent to the propagation of a membrane action potential along the length of the fiber. At present the nature of the coupling between the membrane action potential and activation of the contractile system is unknown. The muscle cell transmembrane potential characteristic of the resting and active states may be recorded by piercing the fiber with a fine microelectrode.[3] A typical record so obtained is shown and explained in Figure 1.

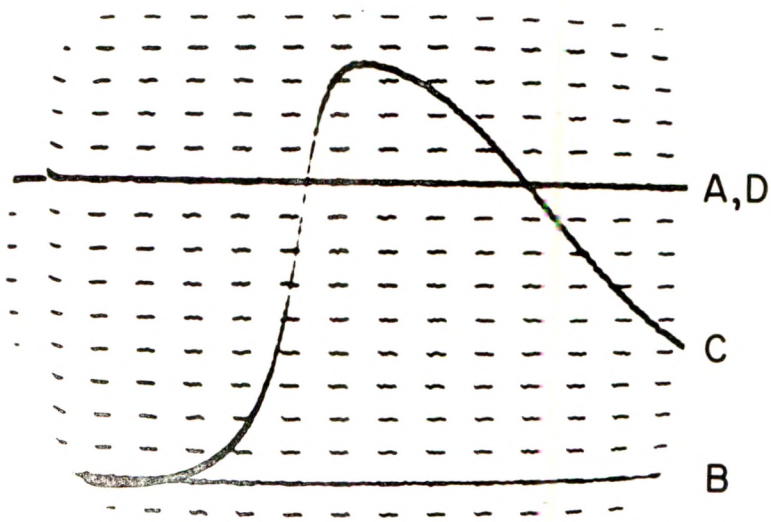


FIG. 1. A typical resting and action potential record obtained distant from the endplate. A and D are records with the microelectrode outside the fiber at the beginning and end of the experiment. B and C are records with the microelectrode inside the fiber. In B the fiber is at rest. C shows the activity which results from stimulation of the motor nerve. Calibration grid; ordinate 10 mv. steps, abscissa 0.2 millisecond intervals. The last portion of the falling phase was not recorded.

Initiation of the Muscle Action Potential. A propagated muscle action potential may be initiated at that point along the length of a muscle fiber where the resting membrane potential is suddenly reduced to a critical value. If the membrane potential does not reach the critical

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level, the disturbance remains localized and the membrane potential returns to the resting level, no propagated action potential being elicited. These facts are illustrated in Figure 2 which shows the membrane potential of a single muscle fiber recorded at the zone of application of a short electrical current of variable intensity.

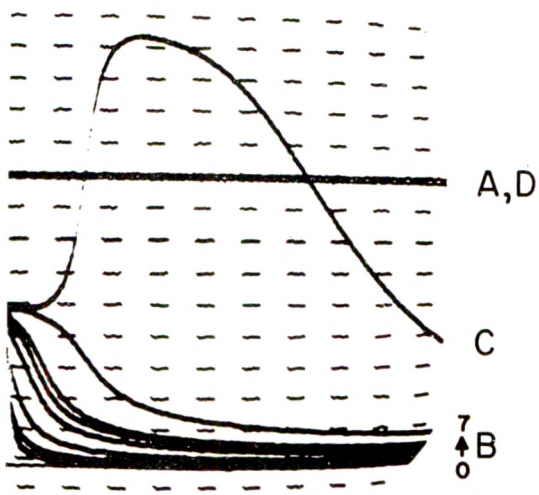


FIG. 2. Reduction in membrane potential recorded in the zone of application of an outwardly directed electric current. A,D and calibration grid as in Fig. 1. B_{1-7}, series of records showing return of the membrane potential toward the resting level of −92 mv. (B_0) immediately after the extrinsic electric current is removed. In B_1 current strength is least, in B_7 greatest. C is a propagated action potential produced by applying current slightly stronger than in B_7. Critical membrane potential at which a propagated action potential is produced is −41 mv.

It can be seen that at the close of a weak stimulus the membrane potential returned to the resting value. A stimulus strong enough to reduce the membrane potential from 92 to 41 mv. produced a critical state whereby a propagated action potential arose.

Changes in membrane potential of a muscle fiber at the endplate occurring during neurogenic initiation of an action potential have been studied using an internal microelectrode.[4] A typical record obtained thereby[4] is shown in Figure 3. Similar results have been reported by

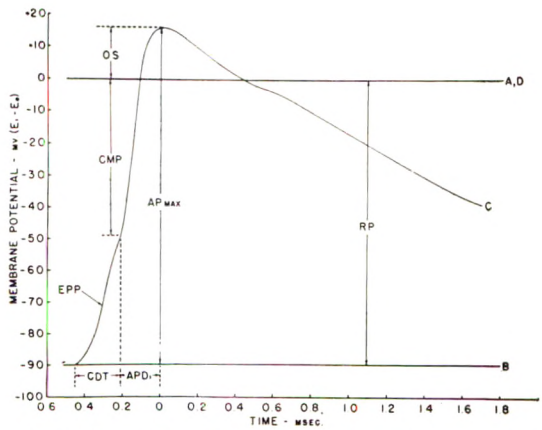


FIG. 4. Plot of a typical resting and action potential record obtained at the center of the endplate to illustrate some of the nomenclature used in this paper. A, B, C and D as in Figure 1. EPP, endplate potential; CMP, critical membrane potential. For further details see reference 4.

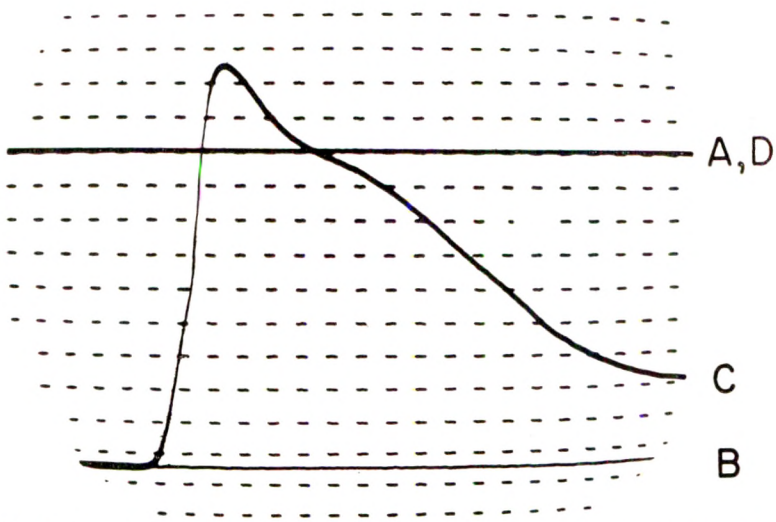


FIG. 3. A typical resting and action potential record obtained at the center of the endplate. A, B, C, D and calibration grid as in Figure 1. Trace C was produced by stimulating the motor nerve. (Rising phase of trace C retouched.)

Fatt and Katz.[5] For *in vivo* neurogenic initiation of muscle action potentials at the endplate, like the electrical initiation just described, it is required that the transmembrane potential be reduced to a critical value.

For descriptive purposes, a typical membrane action potential recorded at the endplate is redrawn in Figure 4. When a nerve action potential arrives, it causes localized reduction of membrane potential at the endplate called the endplate potential (EPP). This record shows that the membrane potential was reduced to the critical level (CMP), and as a consequence a propagated action potential was initiated in the muscle fiber.

As is well known, the principal action of *d*-tubocurarine is to reduce the sensitivity of the endplate to acetylcholine. Therefore if sufficient *d*-tubocurarine is applied to a nerve-muscle preparation, neuromuscular transmission may be blocked, i.e., a nerve action potential will no longer initiate a muscle action potential. Under these conditions, with the intracellular microelectrode at the endplate, an uncomplicated endplate potential is recorded, as shown in Figure 5. These records were obtained by stimulating the nerve at three-second intervals, during which time the endplate was perfused with a minute volume of Ringer's solution in order to remove *d*-tubocurarine.[6] The result was a very prompt increase in the amplitude of the EPP until finally the CMP was reached and thereby a propagated action potential was initiated. Washout of *d*-tubocurarine caused no change in the time course of the EPP, indicating that its removal simply increases the sensitivity of the endplate membrane for acetylcholine. If the washout of *d*-tubocurarine is continued, the EPP rises with greater speed, the CMP is reached earlier and the result is a decrease in transmission delay at the n-m junction.

Membrane Potential Changes Produced by Transient Application of Acetylcholine to the External Surface of the Endplate Membrane. Acetylcholine is thought to react with the endplate membrane causing this structure to increase its permeability to ions. Further information on this point can be gained from studying the reaction under conditions where the extracellular electrolyte composition is varied. Unfortunately, variations in electrolyte usually affect the behavior of the nerve and therefore in such experiments it is necessary that some other means be used to apply small constant amounts of acetylcholine to the endplate. The latter problem has been solved at least partially by development of a method in which acetylcholine is applied electrophoretically.[7] A brief description of the method is as follows: The membrane potential of the muscle cell at the endplate is recorded with an internal

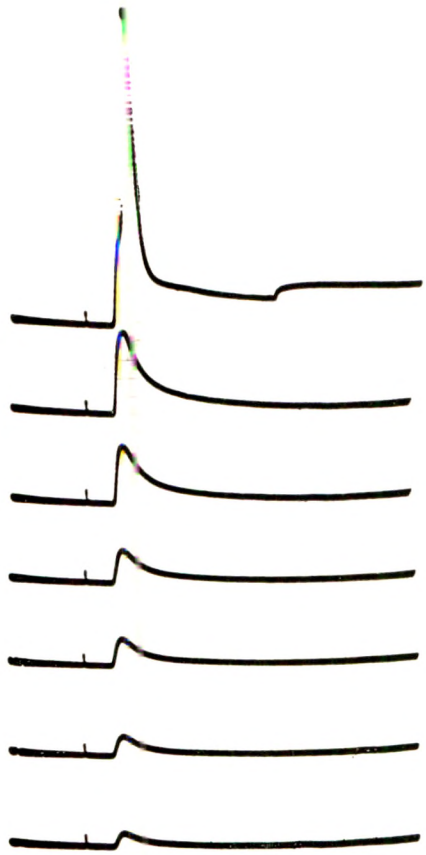


FIG. 5. Serial group of endplate potential records obtained at 3 second intervals during perfusion of a muscle fiber immersed in Ringer's solution plus *d*-tubocurarine, 5.3 mg./L. Perfusion with Ringer's solution began with the record at the bottom.

microelectrode. (Fig. 6.) At a point 10 to 20 μ from the recording site, acetylcholine ions are delivered electrophoretically to the external surface of the endplate. To do this a microneedle is filled with acetylcholine (0.55 M) and contact with the solution is made via an Ag-AgCl electrode. Diffusion of acetylcholine ions is prevented by applying a negative potential to the electrode. Brief ejection of acetylcholine ions occurs when the electrode potential is driven positive by applying a 5 millisecond positive square wave voltage pulse to the system as indicated.

When increasing amounts of acetylcholine ions are applied to the endplate by this method, the membrane potential at this site is progressively reduced, as shown in Figure 7. "Endplate potentials" of increasing magnitude are produced until finally the critical membrane potential is reached and a propagated action potential is elicited. If the amount of applied acetylcholine ions is increased still further, multiple action potentials appear.

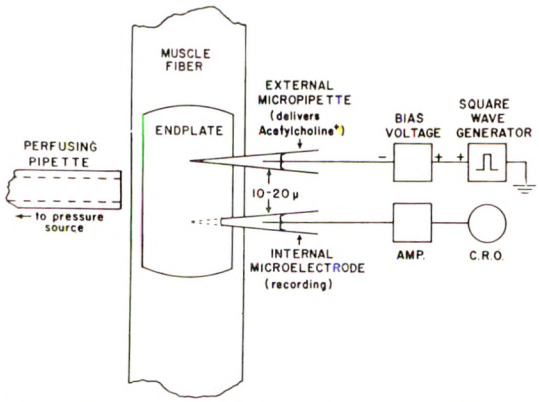


FIG. 6. Schematic diagram showing the experimental arrangement used when recording changes in the transmembrane potential produced during microelectrophoretic application of acetylcholine ions to the outer surface of the endplate. The pipette to the left is used when changing the composition of the extracellular fluid in this zone. For further details see text.

Mechanism by Which Acetylcholine Reduces the Membrane Potential at the Endplate. Any change in membrane potential means that the distribution of electric charge (ions) across the membrane is altered. Reduction of the membrane potential requires ionic redistribution, as stated in Figure 8.

It follows that acetylcholine facilitates transmembranal movement of ions, a process alternatively described as an increase in membrane permeability. A likely source of inward moving positive charge would be Na^+ ions, that is to say, one action of acetylcholine might be to increase the Na^+ ion permeability of the end plate membrane. To test this hypothesis it is necessary to alter the extracellular Na^+ concentration and then measure the change in membrane potential during high speed transient application of a constant amount of acetylcholine to the outer surface of the endplate membrane, using the electrophoretic method.

Two types of experiments were performed.[8] In the first type (Fig. 9) the muscle was placed in normal Ringer's solution and single endplates were perfused with NaCl-free Ringer's solution. Sucrose was substituted to maintain constant osmotic pressure. Before, during and after the perfusion the membrane potential change produced subsequent to electrophoretic application of a small quantity of acetylcholine ions was recorded. It can be seen that removal of NaCl virtually abolishes the response to acetylcholine ions.

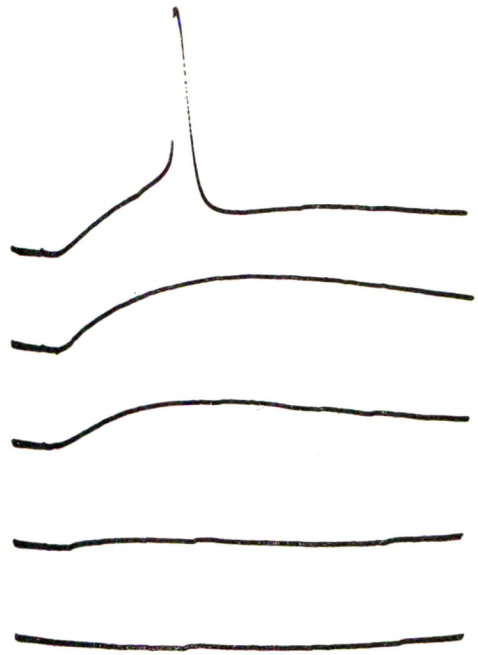


FIG. 7. Endplate potentials produced by microelectrophoretic application of acetylcholine ions to the endplate in successively increased amounts. Intracellular recording.

The second type of experiment (Fig. 10) was essentially like the first except that the muscle was equilibrated in Ringer's solution containing no NaCl and the endplate was perfused with normal Ringer's solution It can be seen that this perfusion restores the response to application of acetylcholine ions. From a series of such experiments it was found that the response of the end-plate membrane to acetylcholine ions is reduced at least by a factor of 14 when extracellular NaCl is removed.

The conclusions one may draw from the preceding experiments are: (1) combination of acetylcholine ions with endplate receptors is inhibited when the total ionic strength of the extracellular solution is reduced; (2) a prominent response of the endplate membrane when

reacted with acetylcholine ions is an increase in its permeability to Na^+ ions.

At present there is no evidence bearing on the validity of the first possible conclusion. This is a critical point which merits further investigation. It must also be pointed out that the increase in average, 5×10^{-16} equivalents of acetylcholine ions are applied to the endplate.[7] Since the application is a wasteful process, the minimum amount of acetylcholine ions required must be even smaller. Fatt and Katz[5] have shown that the net charge transported across the

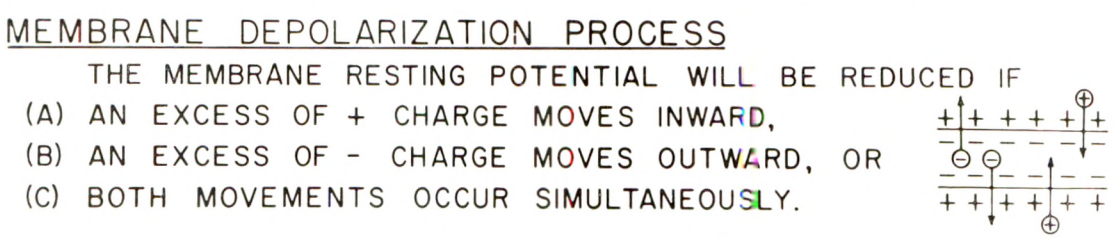


FIG. 8. Membrane depolarization process



FIG. 9. Typical endplate potential changes produced by microelectrophoretic application of acetylcholine ions to the endplate of a single muscle fiber. The fiber was immersed in normal Ringer's solution and perfused with "sucrose Ringer's" solution (NaCl-free). Peak amplitudes of the endplate potentials were as follows: before perfusion 24 mv., during perfusion less than 1 mv., after perfusion 23 mv. Resting potentials measured during these periods were 90, 89 and 90 mv.

FIG. 10. Typical endplate potential changes produced by microelectrophoretic application of acetylcholine ions to the endplate of a single muscle fiber. The fiber was immersed in "sucrose Ringer's" solution (NaCl-free) and perfused with normal Ringer's solution. Peak amplitudes of the endplate potentials were as follows: before perfusion 1 mv., during perfusion 18 mv., after perfusion 2 mv. Resting potentials measured during these periods were 87, 80 and 86 mv.

permeability brought on by acetylcholine ions is not selectively limited to Na^+ ions but appears to include the K^+ ion as well. For further evidence on this matter see references 2, 5 and 9.

"Trigger Function" of Acetylcholine. As stated previously, the genesis of the endplate potential depends at least in part on the penetration of the membrane by positively charged extracellular ions. Released acetylcholine ions might act as a source of penetrating positive charge but it is a fact that the total electrical charge which moves is much too great to be entirely accounted for in this way. To illustrate, by means of microelectrophoresis it can be shown that a propagated action potential may be elicited if, on the membrane during a subthreshold endplate potential is 80×10^{-16} equivalents, and in the absence of *d*-tubocurarine the figure increases to 200 to 400 $\times 10^{-16}$ equivalents. Hence, each acetylcholine ion facilitates transmembranal movement of at least 20 to 75 additional univalent ions and in this sense it may be thought of as having a "trigger function."

Release of Acetylcholine. The mechanism by which acetylcholine ions are released during the "resting" state and subsequent to arrival of a motor nerve impulse has been the subject of intensive investigation by Katz and his collaborators. This important work cannot be discussed here and the reader is referred to the

reviews of Fatt,[1] and Castillo and Katz[2] which deal more extensively with the subject.

Acknowledgment: The work of this laboratory was aided by a grant from Hoffmann-La Roche, Inc, Nutley, N. J.

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Neuromuscular Blocking Agents*

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SINCE 1948 the investigation of neuromuscular blocking agents has been in a turmoil. The careful studies of Paton and Zaimis[1] on decamethonium and homologues showed that the paralysis produced by these agents was different from the paralysis produced by tubocurarine. Even though the blockade produced by decamethonium was limited to the neuromuscular junction, it was found that neostigmine did not antagonize the block; in fact in some instances it would make it worse. Burns and Paton[2] observed that the blockade produced by decamethonium coincided with a sustained depolarization of the endplate region. Since tubocurarine does not produce such a depolarization, we can classify neuromuscular blocking agents as *depolarizing neuromuscular blocking agents* and *non-depolarizing neuromuscular blocking agents*. Many statements have appeared in the literature that substances are depolarizing neuromuscular blocking agents without direct evidence of depolarization of the endplate region. The indirect evidence is based on effects such as spastic paralysis in the pigeon, contracture of the rectus abdominis of the frog, lack of antagonistic effect with neostigmine, and the height of contraction during and after tetanic stimulation. All these are useful tools, but the ultimate classification, whether an agent blocks by depolarizing or without depolarizing, lies in recording the depolarization and comparing this with the observed neuromuscular blockade. In our laboratory we used, slightly modified, the technic described by Burns and Paton.[2] A moving electrode scans the surface of a muscle and the potential between the moving electrode and a stationary electrode placed near the tendon of the same muscle is recorded. The results lead to the following classifications: (1) Depolarizing neuromuscular blocking agents—decamethonium, succinylcholine, succinylmonocholine, neostigmine and edrophonium (tensilon®); (2) Non-depolarizing neuromuscular blocking agents—tubocurarine, dimethyl-tubocurarine, flaxedil,® mytolon® and 1 : 5 di (N-allyl-N-di-methylaminophenyl)pentan-3-one(53–67).

In the list of depolarizing neuromuscular blocking agents succinylmonocholine is of interest because it is the first degradation product of succinylcholine[3] and is still an effective depolarizing neuromuscular blocking agent, a fact which should not be overlooked by those who use succinylcholine.

Neostigmine, edrophonium and (53–67) are primarily cholinesterase inhibitors and as such are antagonists to tubocurarine. In larger doses all three can produce neuromuscular blockade. With neostigmine and edrophonium this blockade is accompanied by a sustained depolarization whereas with (53–67) no such depolarization is evident.

Zaimis[4] compared the paralyzing dose of decamethonium and of tubocurarine in different species. If the species were arranged so that the paralyzing dose of decamethonium increased when going toward the right, the paralyzing dose of tubocurarine tended to decrease in that same direction. When we compare different muscles of the same species, e.g., the soleus and tibialis of the cat, we find that the paralyzing dose of decamethonium is smaller for the tibialis than for the soleus whereas with tubocurarine the opposite is true.[5] In Figure 1 an attempt is made to explain the different behavior of the soleus and tibialis muscles. Although it is oversimplified, this simple hypothetic graph helped me very much in tying controversial facts together. The distance from top to bottom represents the membrane potential. The dashed line arbitrarily placed in the middle represents the critical membrane potential. This is the membrane potential at which, after quick depolarization, an action potential ensues. I should like to assume that a sustained depolarization at this level will produce blockade.

After stimulation of the sciatic nerve acetyl-

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choline is released at the endplate region and, according to our present concepts, this acetylcholine gives rise to an endplate potential (e.p.p.). In Figure 1 I propose that the resulting e.p.p. is different in the two muscles. An important factor may be the relative relation between the e.p.p.'s. In other words, the sustained level of depolarization produced by a certain dose of decamethonium is lower in the soleus than in the tibialis. In the tibialis the sustained depolarization would reach the critical membrane potential and thus no action potential or twitch could

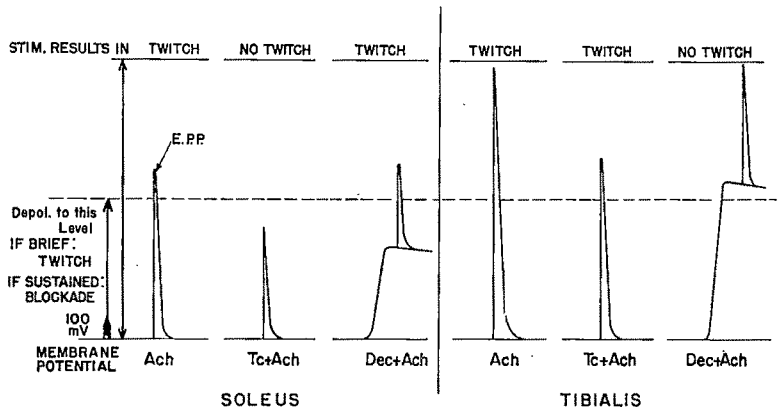


FIG. 1. For explanation see text.

the heights of the e.p.p. and the critical membrane potential; this is shown in Figure 1. Another important factor may be the angle at which the rising phase of the e.p.p. reaches the critical membrane potential.

What happens after the administration of tubocurarine? The competitive antagonism between acetylcholine and tubocurarine will prevent a certain portion of the acetylcholine from reacting with the receptive substance and the result is a smaller e.p.p. Let us assume that the e.p.p. is decreased to two-thirds its original height. In the soleus muscle this depolarization is, according to our hypothetic scheme, not enough to reach the critical membrane potential and thus tubocurarine prevents the production of an action potential or twitch. In the tibialis, however, two-thirds the original e.p.p. still reaches the critical membrane potential and thus the action potential or twitch is still present. In order to produce paralysis of the tibialis we have to increase the dose of tubocurarine so that the e.p.p. may be diminished to half its original height. In other words, as far as tubocurarine is concerned the paralyzing dose for the tibialis is higher than that for the soleus.

What about decamethonium? Here the sustained level of depolarization is important. Let us now assume that the ratio of the depolarization produced by decamethonium in the two muscles is the same as the ratio of the heights of occur. In the soleus, on top of the sustained depolarization, the e.p.p., even though it is diminished, reaches the critical membrane potential; and since this is a quick depolarization, we will observe a normal twitch. In order to produce paralysis with decamethonium in the soleus we have to increase the dosage. Thus, the paralyzing dose of decamethonium for the soleus is higher than that for the tibialis.

The e.p.p.'s after decamethonium are drawn smaller than the original e.p.p.'s because I believe that besides the depolarization produced by decamethonium there exists also a mutual antagonism between acetylcholine and decamethonium similar to that proposed for tubocurarine.

We observed differences between decamethonium and tubocurarine. Are there similarities in their effects?

Frequency of Stimulation. Increased frequency of stimulation of the sciatic-gastrocnemius preparation of the rat results in a decrease of the paralyzing dose for both depolarizing and nondepolarizing neuromuscular blocking agents.[6]

Neostigmine Antagonism. (1) In the isolated lumbrical muscle of the rabbit Jenden et al.[7] described two phases of paralysis after the administration of decamethonium. The first phase has a rapid onset, is short-lasting and cannot be antagonized by neostigmine. This is the usual blockade seen after decamethonium in

contrast to the second phase. The second phase is slow in onset, outlasts the first phase and can be antagonized by neostigmine. (2) In the intact cat Jewell et al.[8] found that after two or three injections of decamethonium the neuromuscular blockade in the soleus can be antagonized by neostigmine. (3) In some myasthenia gravis patients Churchill-Davidson et al.[9] observed that paralysis produced by decamethonium was antagonized by neostigmine.

In terms of Figure 1 all these facts can be explained if we suppose that the safety factor, i.e., the difference between the e.p.p. and the critical membrane potential, is diminished. This could be caused by a lack of free acetylcholine, a lack of free receptive substance, an inability of the endplate region to undergo adequate depolarization or a change of the critical membrane potential. In more general terms, we can try to formulate what occurs at the endplate with the aid of Figure 2. I have assumed a reversible reaction between acetylcholine and the receptive substance which leads to the formation of AchR. The amount of AchR produced depends on the concentration of the free receptive substance and the free acetylcholine. Also, AchR is responsible for the local depolarization. When Ach comes in a single burst from the nerve impulse, AchR is short-lived and results in a quick depolarization and a quick repolarization, better known as the endplate potential. Under special circumstances it is possible to maintain a large amount of AchR at the endplate. The result then is a sustained depolarization which in itself produces neuromuscular blockade similar to the one seen after administration of decamethonium.

Tubocurarine and acetylcholine are both assumed to compete for the same free receptive substance. Therefore in the presence of tubocurarine the acetylcholine released after a nerve impulse will find a smaller concentration of free receptive substance. The result is that less AchR is formed and there is therefore less depolarization at the endplate. The combination of tubocurarine and receptive substance does not lead to depolarization, and hence tubocurarine produces its block by decreasing the total amount of free "R" available for acetylcholine.

With decamethonium the picture is slightly more complex. Let us assume that decamethonium reacts with the receptive substance in the same way as does acetylcholine. It will therefore occupy a certain amount of the free receptive substance, leaving a smaller amount available to react with acetylcholine. DecR, however, by itself produces a depolarization of the endplate. Under special circumstances this depolarization produced by DecR is quick enough to result in a twitch. DecR is ordinarily long-lasting (that is, more than one minute)

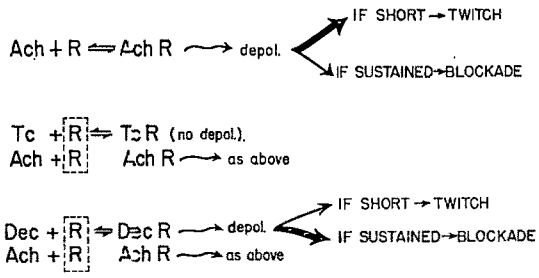


FIG. 2.

and the sustained depolarization which it produces is the primary cause of neuromuscular blockade seen after administration of decamethonium. One can, however, theorize that at certain occasions or in special motor endplates the sustained depolarization produced by DecR is not the primary cause of blockade. If motor endplates exist in which the depolarization from the maximal amount of AchR after maximal stimulation is barely able to reach the critical membrane potential, then one may postulate that in these same muscles the concentration of free receptive substance is a very important factor for neuromuscular transmission. Thus, these muscles will be very sensitive to tubocurarine. Furthermore, if the maximal amount of AchR is relatively ineffective in these muscles in producing depolarization adequate to stimulate, we can assume that DecR is equally ineffective in producing the sustained depolarization necessary to block. Therefore, it will require a large amount of decamethonium to produce a depolarizing neuromuscular blockade, so large indeed that before this level of sustained depolarization is reached the production of sufficient quantities of AchR after nerve stimulation is interfered with. This then becomes the cause of neuromuscular blockade despite the presence of a slight sustained depolarization. Under these circumstances neostigmine becomes effective as an antagonist, since by preventing the destruction of acetylcholine it will allow a larger concentration of acetylcholine to react with the receptive substance and thus neuromuscular transmission can be restored.

This discussion is much theory based on very

few facts. I hope that this theorizing may open the road for many new experiments.

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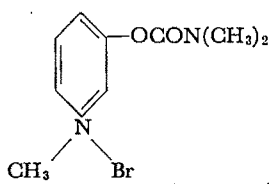
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Pharmacology of the Anticholinesterase Drugs—Mestinon, Prostigmin, Tensilon and TEPP*

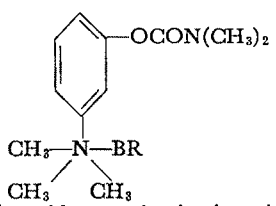
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Nutley, New Jersey

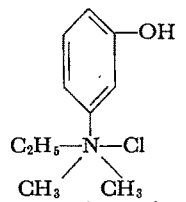
MESTINON® is one of the new anticholinesterase drugs which is being tested currently in myasthenia gravis. A pyridine analog of prostigmin, mestinon was first synthesized by Urban and Schnider (1945)[12] in the Hoffmann-La Roche Laboratories at Basle, Switzerland. It had been found by Osserman et al.[8] and Tether[11] in 1954 to have advantages over prostigmin® in duration of action and mildness of side effects. This paper is concerned with the pharmacology of mestinon in comparison with prostigmin, tensilon® and TEPP (tetraethyl pyrophosphate). Prostigmin has long been the anticholinesterase of choice in the treatment of the disease. Tensilon is a short-acting anticholinesterase which is used as a diagnostic agent (Osserman and Kaplan[7]). TEPP is a representative of the long-acting anticholinesterases which have had unsuccessful trials in the disorder (Grob and Harvey[5]).



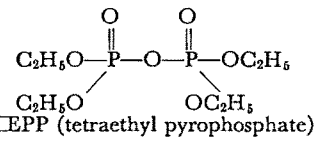
Mestinon bromide; pyridostigmine bromide (dimethyl carbamic ester of 3-hydroxy-1-methylpyridinium bromide)



Prostigmin bromide; neostigmine bromide (dimethyl carbamic ester of 3-hydroxy-phenyl-trimethyl ammonium bromide)



Tensilon chloride; edrophonium chloride (3-hydroxy-phenyl-ethyl-dimethyl ammonium chloride)



TEPP (tetraethyl pyrophosphate)

Toxicity. The acute toxicity of mestinon in comparison with prostigmin was worked out in detail by Fromherz and Pellmont[4] in 1953.

TABLE I
ACUTE TOXICITY IN MICE

	Intravenous		Subcutaneous		Oral	
	LD_{50} (mg./kg.)	Ratio	LD_{50} (mg./kg.)	Ratio	LD_{50} (mg./kg.)	Ratio
Mestinon	1 37	1	2.7	1	32	1
Prostigmin	0 17	8	0.42	6	7.5	4
Tensilon	2 0	1/6	130	1/50	600	1/20
TEPP	0 027	50	.....	...	.....	...

The average toxicity of mestinon was one-fifth that of prostigmin by various routes in mice, rats, rabbits, guinea pigs and cats. The relative toxicities varied from about equal toxicity of mestinon and prostigmin by the subcutaneous route in mice and the oral route in rats to one-fifteenth by the subcutaneous route in rats. The relative toxicities as measured in our laboratory are shown in Table I. Our data are in agreement

* From the Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey

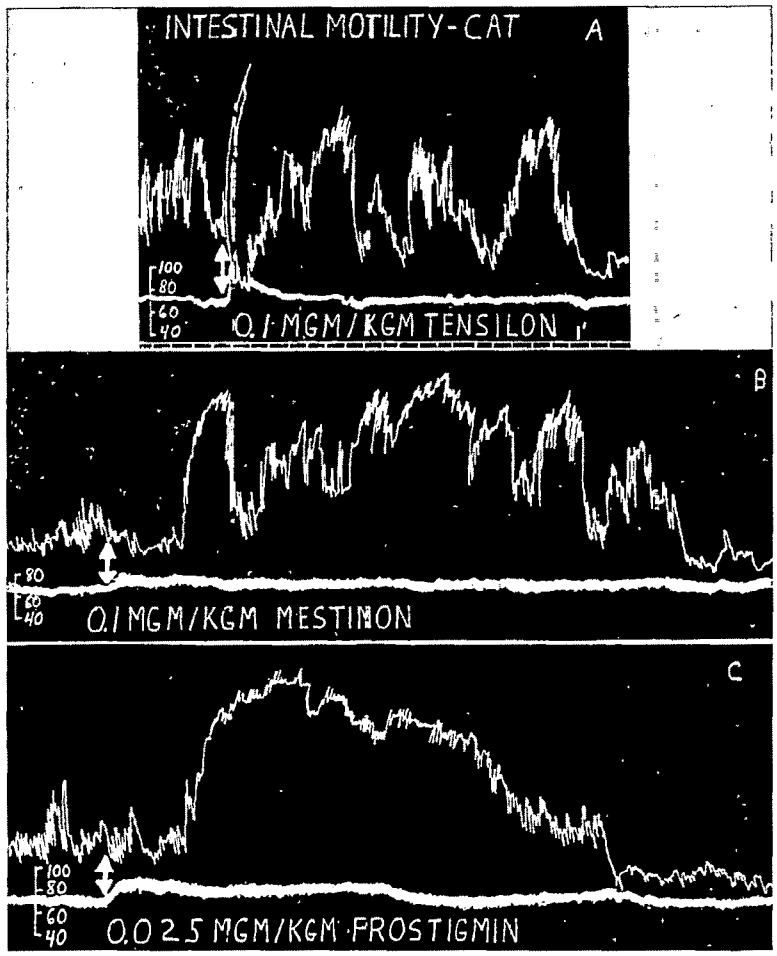


FIG. 1. Comparative stimulating effects of tensilon, mestinon and prostigmin on the intact intestine of the cat. Motility was recorded with a tambour connected by a glass tube to the lumen of the duodenum. Top curve, intestinal motility; middle curve, arterial pressure.

with those of Fromherz and Pellmont (1953) in that mestinon has been found to be one-fourth to one-eighth as toxic as prostigmin. Tensilon is one-sixth to one-fiftieth as toxic as mestinon, while TEPP is fifty times more toxic than mestinon. All the compounds produce typical signs of over-stimulation by acetylcholine—salivation, increased intestinal motility, defecation, fibrillation, convulsions and respiratory paralysis.

Intestinal Motility. Mestinon has about one-fourth to one-half the potency of prostigmin as a stimulant of isolated rabbit intestine (Fromherz and Pellmont[4]). It sensitizes the isolated intestine to acetylcholine and the effects are blocked by atropine. The intact rabbit intestine is also stimulated by mestinon administered intravenously at doses two to four times those of prostigmin and the effects of acetylcholine are potentiated. The intact intestine of the cat also is stimulated by this dosage. In Figure 1 it is shown that 0.1 mg./kg. of tensilon administered intravenously produces a very rapid stimulation followed quickly by relaxation. Mestinon and prostigmin stimulate after a delay of several minutes. Mestinon is about one-fourth as active as prostigmin and the duration of action is at least as long. TEPP has a potency similar to that of prostigmin but the duration of action is longer.

Effect on Blood Pressure. Fromherz and Pellmont[4] in 1953 reported that mestinon and prostigmin have very little effect on blood pressure of cats in small non-toxic doses. Large

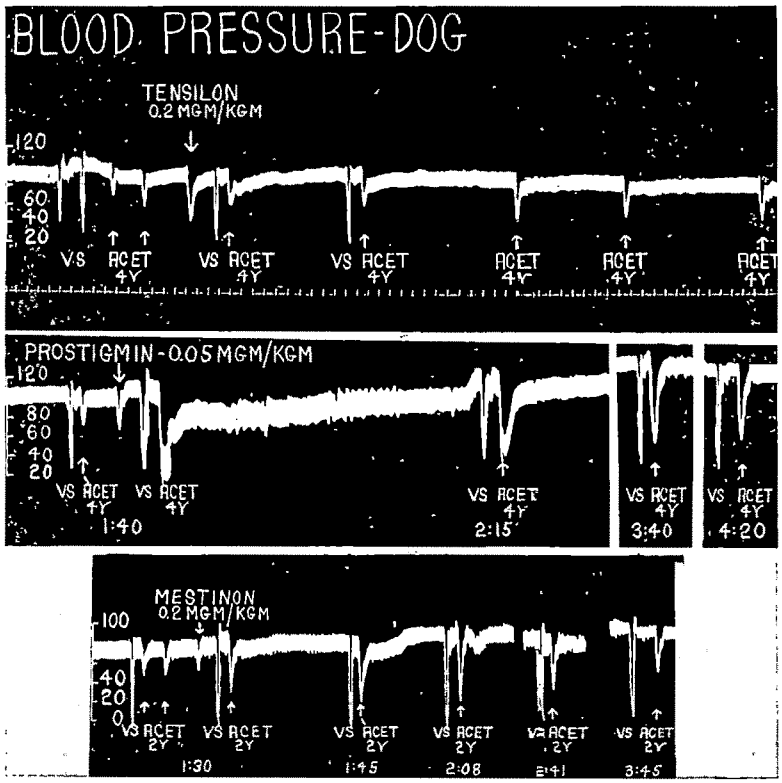


FIG. 2. Potentiation effects of tensilon, mestinon and prostigmin on the responses of the blood pressure to acetylcholine and vagus stimulation. Blood pressure was measured from the carotid artery of a dog anesthetized with pentobarbital.

doses cause a fall of blood pressure as a result of parasympathetic stimulation. This is accompanied by dilation of peripheral vessels. Hobbiger[6] in 1950 found that mestinon is one-fifth as active as prostigmin as a sensitizer of the frog rectus to acetylcholine while TEPP is equal to prostigmin.

Our experience confirms the slight effects of mestinon on the blood pressure of cats. (Fig. 1.) The results of potentiation experiments on acetylcholine and the vagus in the dog are shown in Figure 2. Tensilon potentiates the depressor effects of acetylcholine for about fifteen minutes but the depressor response to vagus stimulation is not potentiated. Mestinon and prostigmin potentiate both effects for several hours. Mestinon is not more than one-fourth as active as prostigmin. There is a prolonged potentiation of these effects by TEPP. This compound is a stronger cholinergic agent than prostigmin and is also much more toxic.

Neuromuscular Transmission. Fromherz and Pellmont[4] in 1953 found that mestinon has about one-half of the anticurare activity of prostigmin in cats. The paralysis of the sciatic tibial preparation produced by tubocurarine was antagonized by 0.2 mg./kg. of mestinon or 0.1 mg./kg. of prostigmin. Casier and Verbeke[3] in 1950 also observed anticurare action in the same dose range. Blaschko et al.[1] in 1949 used the rat phrenic diaphragm preparation and found that mestinon was $\frac{1}{40}$ as strong as prostigmin. Hobbiger[6] in 1950 reported results indicating that the effect of mestinon as an anticurare agent was one-fifth as strong as prostigmin on the frog rectus. TEPP was equal to prostigmin.

Our experiments (Fig. 3) show that mestinon has about one-fourth the activity of prostigmin as an antagonist to tubocurarine paralysis of the sciatic nerve tibial muscle preparation in cats. Tensilon is as active as mestinon but TEPP is weaker. Tensilon has a rapid onset and a short duration of action whereas mestinon, prostigmin and TEPP have long duration and slow onset.

The duration of action in these experiments was estimated from the time required for repeated doses of tubocurarine to reproduce the original block. While tensilon effects were

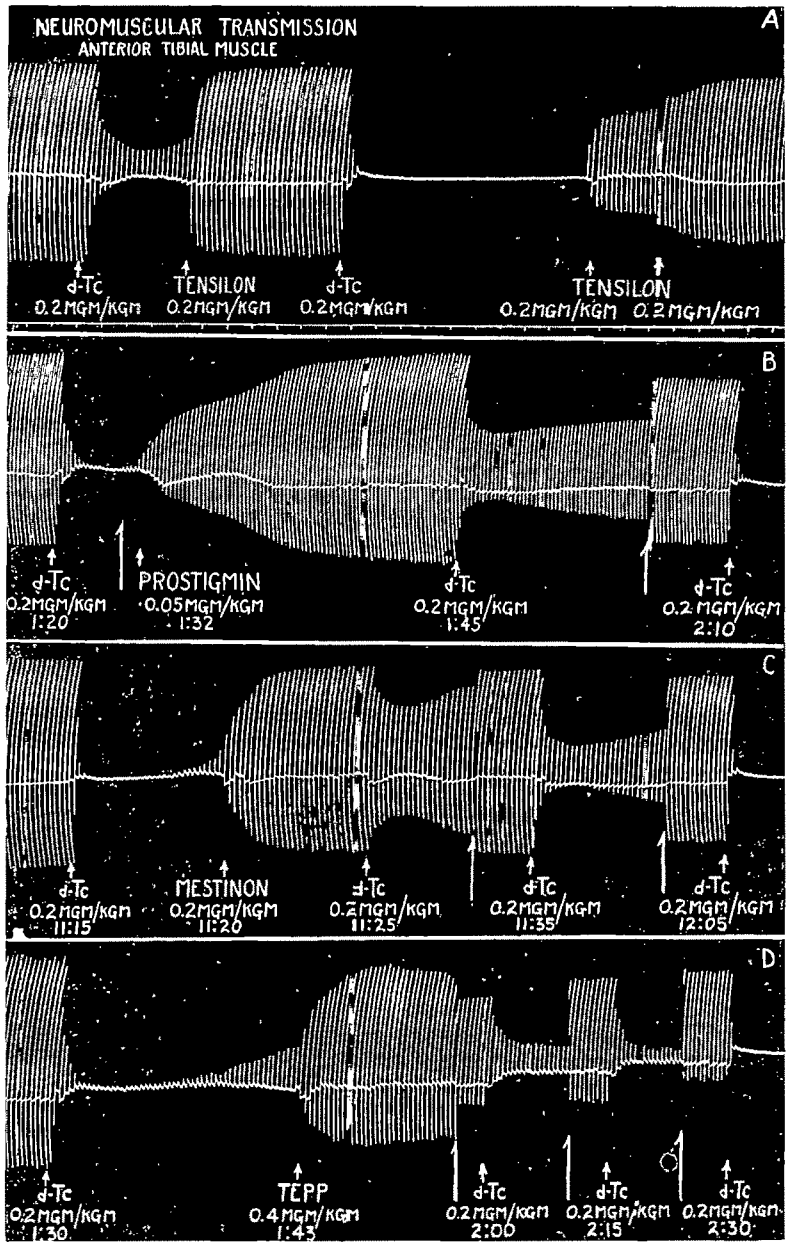


FIG. 3. Anticurare action of tensilon, prostigmin, mestinon and TEPP in a cat anesthetized with pentobarbital. Recording of the anterior tibial muscle stimulated through the sciatic nerve; time is in minutes. At the long arrows, part of the record was omitted.

counteracted by *d*-tubocurarine within ten minutes, the action of mestinon, prostigmin and TEPP lasted up to an hour.

Potentiation of the twitch tension by tensilon is also a rapid action of short duration. (Fig. 4.) Mestinon and prostigmin have a greater delay of onset and a gradual effect on twitch tension. Mestinon is about one-eighth as strong as prostigmin in this test and the duration of effects are about equal. TEPP is relatively weak and it has a much delayed onset of action. The spread between the doses which produces an increase in tension and the curariform effect also differ. This spread between the potentiating dose and the curarizing dose represents the safety margin of the drugs. Tensilon has an eight-fold safety

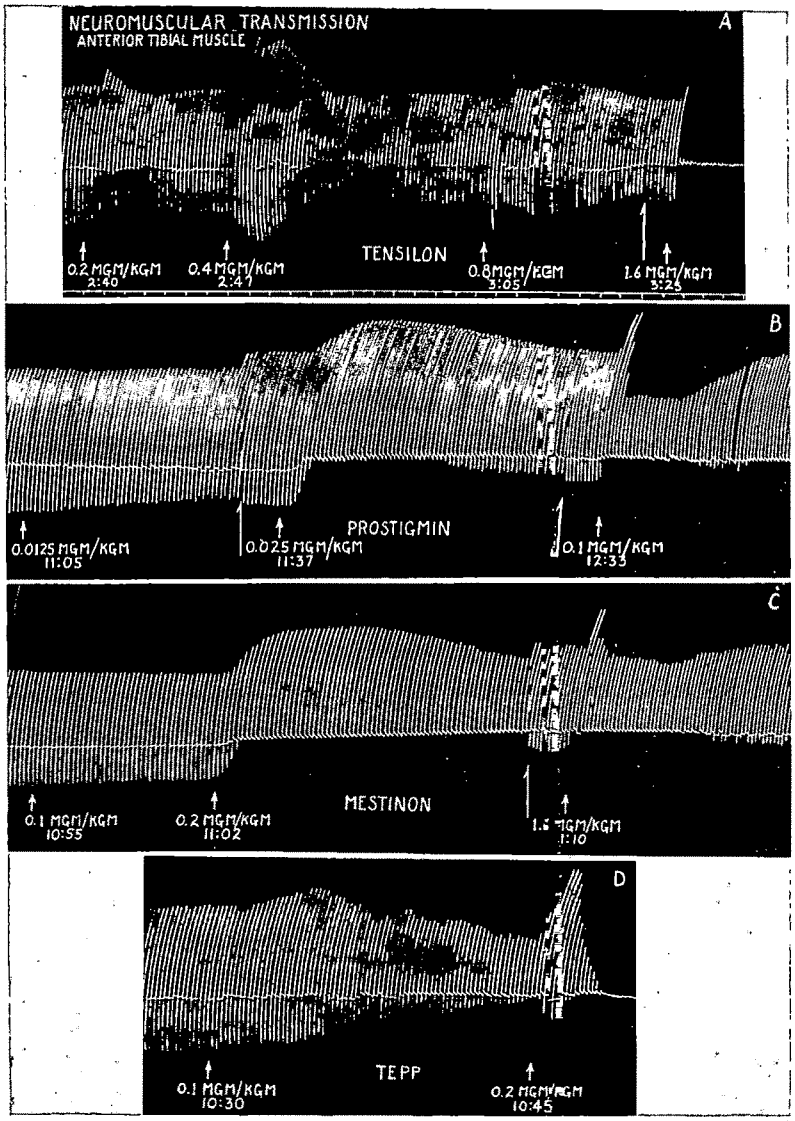


FIG. 4. Potentiation of the twitch tension in cats by tensilon, prostigmin, mestinon and TEPP. Recording of the response of the tibial muscle to stimulation of the sciatic nerve.

margin, prostigmin a four-fold safety margin, mestinon at least an eight-fold safety margin, while TEPP has only a two-fold safety margin. Mestinon has a greater safety margin than prostigmin in this test.

The differences in rate of onset of action in these experiments may represent the differences in the rate of penetration or diffusion to the site of action at the neuromuscular junction. The increase in tension produced by the agents as well as the curariform effect are interpreted as the result of the accumulation of acetylcholine at the sites of action. Alternate explanations for the fast action of tensilon are that it may have a direct depolarizing action at the neuromuscular junction and it may potentiate acetylcholine directly. However, most workers now believe that the action is primarily an anticholinesterase effect with resultant accumulation of acetylcholine. This subject has been reviewed by Riker[10] (1953) and Randall and Jampolsky[9] (1953).

Anticholinesterase Activity. The anticholinesterase activity of mestinon was found to be one-tenth that of prostigmin by Blaschko et al.[1] in 1949. This relative activity was measured *in*

vitro using true cholinesterase from the brain of the dog. The relative anticurare activity on the phrenic diaphragm of the rat was one-fortieth. Hobbiger[6] in 1950 indicated that mestinon had about one-fifth the activity of prostigmin in inhibiting the cholinesterase of frog muscle.

TABLE II
CORRELATION OF CHOLINESTERASE INHIBITION WITH TOXICITY AND ACTIVITY

	Cholinesterase Inhibition		Relative Toxicity in Mice (intravenously)	Relative Anticurare Activity
	Concentration for 50% Inhibition	Ratio		
Prostigmin..	1×10^{-7}	1	1	1
Mestinon...	2×10^{-6}	1/20	1/8	1/4
Tensilon....	2×10^{-5}	1/200	1/50	1/4
TEPP.....	5×10^{-9}	20	6	1/8

This same ratio of activity was found for the sensitizing of muscle to acetylcholine and for the anticurare effect on frog muscle. Casier[2] in 1950 also reported on low anticholinesterase activity of mestinon with respect to true cholinesterase.

Our results indicate that mestinon has about 1/20 the activity of prostigmin when tested on red cell esterase; the activity of tensilon was 1/200 of prostigmin, while TEPP was twenty times that of prostigmin. These relative anticholinesterase values correlate reasonably well with the relative toxicities of the compounds in mice. This indicates that toxicity is a consequence of the cholinergic effects of anticholinesterase agents. The relative anticurare activities do not correlate with the anticholinesterase values or the toxicity. Mestinon itself does show a slightly better ratio of anticurare activity to toxicity and anticholinesterase activity than prostigmin. Tensilon shows a high anticurare activity in relation to anticholinesterase activity and toxicity, while TEPP has a very poor ratio of anticurare activity to toxicity and anticholinesterase activity. The anticurare activities are better correlated with the clinical results than either toxicity or anticholinesterase activities. (Table II)

These anticurare results correlated with the clinical findings in myasthenia gravis reveal that mestinon is better tolerated than prostigmin although it is a weaker drug. Tensilon is very well tolerated and it is quite safe in myasthenia gravis; its very short action is useful for diagnostic purposes. TEPP is useless because of its low margin of safety.

SUMMARY

Mestinon is a pyridine analog of prostigmin which has one-eighth to one-fourth the toxicity of prostigmin, one-fourth the intestinal stimulating capacity, one-fourth the potentiating effects on acetylcholine and the vagus, one-fourth the anticurare activity, one-eighth the potentiating action on twitch tension and one-twentieth the anticholinesterase activity on red cell esterase. Mestinon and prostigmin have a slow onset of action in various tests and a long duration of action in contrast to the rapid onset and short duration of action of tensilon. Mestinon and prostigmin have a much better safety margin between anticurare activity and toxicity than TEPP. The anticurare activity in cats offers a better index of predictability of clinical activity in myasthenia gravis than the toxicity or anticholinesterase activity of these compounds.

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Electromyographic Changes in Myasthenia Gravis*

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A COMPLETE description of the partial block in neuromuscular transmission in myasthenia gravis is a prerequisite in any attempt to uncover the underlying cause of this block. The purpose of this study is to describe in some detail the properties of the myasthenic neuromuscular block. This was accomplished by recording the electrical response of muscle to various patterns of nerve stimulation.

The ulnar nerve was stimulated percutaneously just above the elbow with a rectangular current pulse of 150 microseconds' duration. Great care was taken to insure maximal nerve stimulation at all times. The electrical activity of the muscle was recorded between a surface electrode over the belly of the abductor of the fifth finger and an indifferent electrode. These muscle action potentials were amplified and photographed from the face of a cathode-ray oscillograph. Such action potentials are polyphasic and represent a summation of the individual muscle fiber action potentials in the region of the recording electrode. (Fig. 1.) Thus when some neuromuscular junctions are blocked their individual muscle fibers do not respond to the nerve stimulus and the summated or integrated action potential decreases in size. Contrariwise, when there is a decrease in neuromuscular block more individual muscle fibers respond, and the integrated action potential increases in size. Changes in the size of the integrated action potential therefore serve as a measure of changes in neuromuscular transmission.

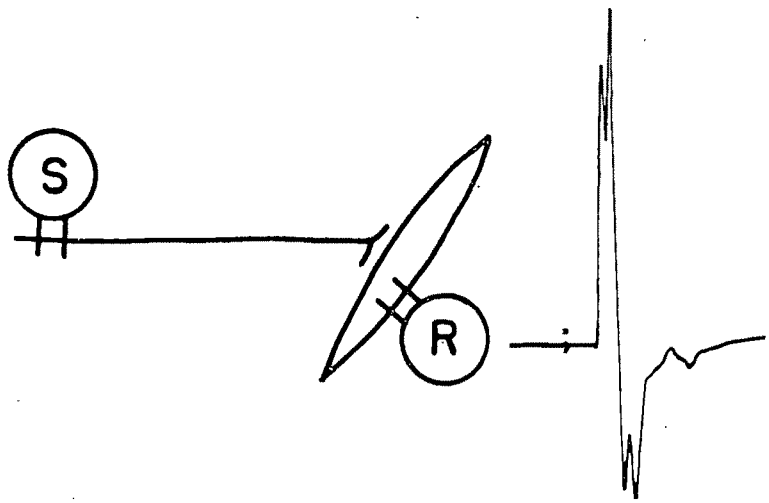


FIG. 1. Diagram of stimulation of the nerve and recording from the muscle as well as a photograph of a typical muscle action potential.

Is there a partial block of the transmission of a single impulse in generalized myasthenia gravis? This question was investigated by comparing the size of the action potential in response to a single nerve stimulus in a group of normal subjects and a group of myasthenic patients. The mean amplitude in a group of twenty normal subjects was 7.5 millivolts; in a group of twenty-six patients with generalized myasthenia gravis it was 5.4 millivolts. While the difference be-

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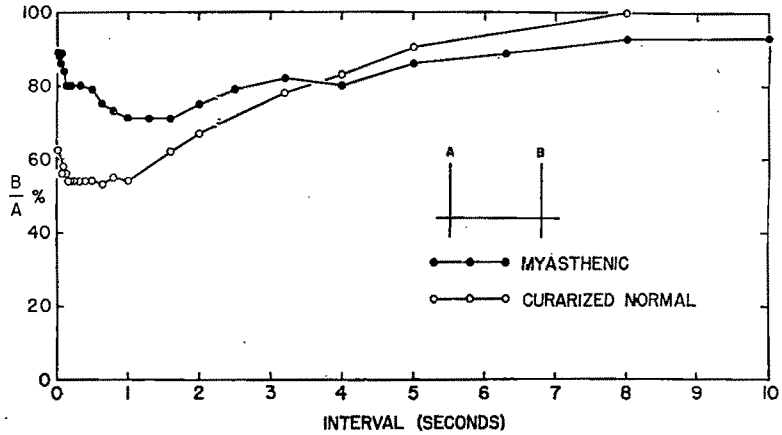


FIG. 2. Time course of the increased degree of block following passage of a single impulse in generalized myasthenia gravis (closed circles) and partial curarization (open circles). The size of the test response (B) is expressed as a percentage of the conditioning response (A) and is plotted against the interval between the responses.

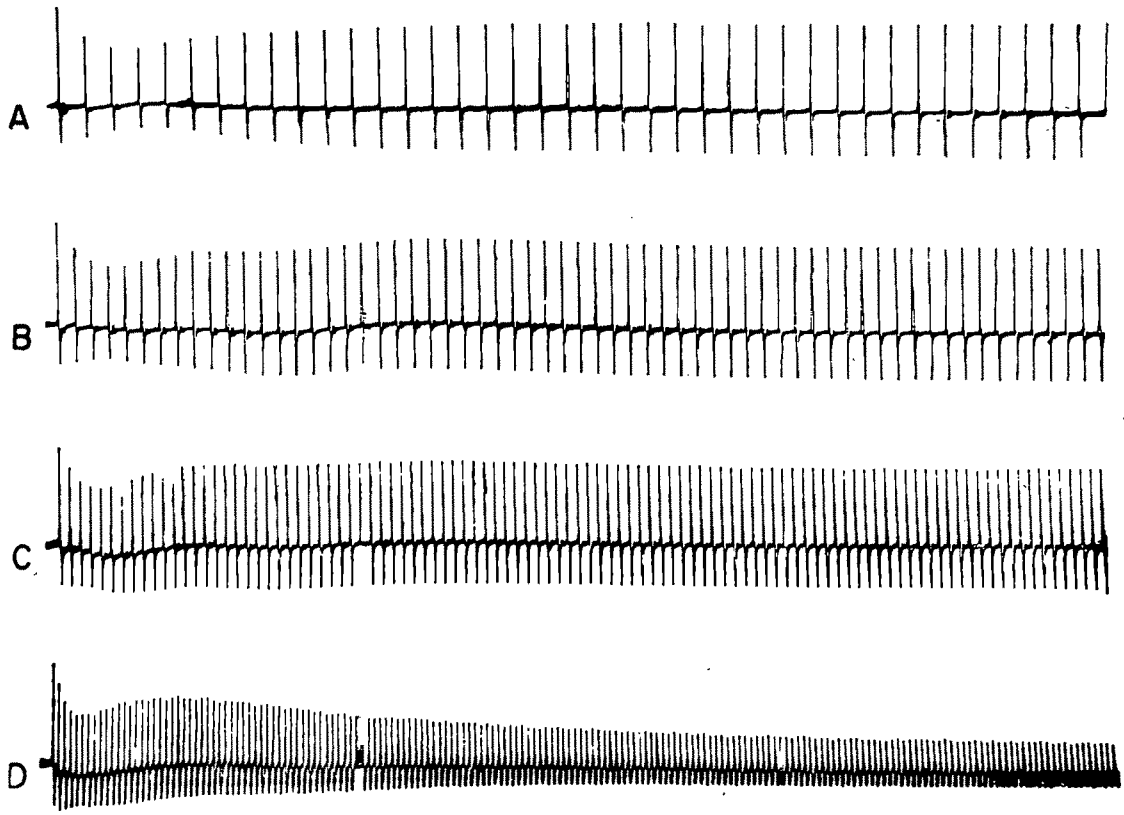


FIG. 3. Action potential response to repetitive nerve stimulation in generalized myasthenia gravis. Stimulation at rates of A, 5 per second; B, 10 per second; C, 25 per second, and D, 50 per second. The late exponential increase in degree of block has not yet occurred in the segments of the record shown in A and B.

tween these means was statistically significant, there was a wide overlap between the individual values in the two groups. Thus statistical analysis suggests that there is a partial block of the transmission of a single impulse in the myasthenic group but it provides no information regarding the presence or absence of block in many of the individual myasthenic patients whose potentials were within the broad normal range. The fact that neostigmine can increase the size of the action potential response to a single stimulus in most patients with generalized myasthenia gravis, even when the potential is within the normal range, may be taken to indicate that some degree of block was present in these patients.

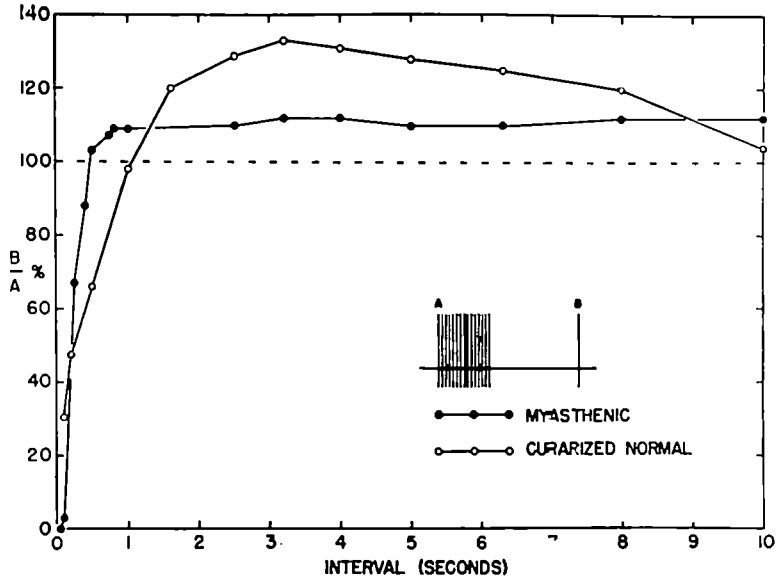


FIG. 4. The time course of the post-tetanic facilitation of neuromuscular transmission in generalized myasthenia gravis (closed circles). The conditioning tetanus was a train of 121 stimuli at 200 per second. The size of the test response (B) is expressed as a percentage of the size of a pretetanic response (A). This is plotted against the interval between the end of the conditioning train and the test stimulus. Similar results are obtained in a partially curarized normal subject (open circles).

The effect of transmission of a single impulse on the ability of the myasthenic neuromuscular junctions to transmit a subsequent impulse was next investigated. A conditioning nerve stimulus was followed by a test stimulus and the test action potential response was compared to the conditioning response. In patients with generalized myasthenia gravis the second (test) response was smaller than the first (conditioning) response, demonstrating that there was an increased degree of block to the passage of the second response. The time course of this increased block following the passage of a single impulse was traced out by varying the interval between conditioning and test stimuli. (Fig. 2.) The block increased following the passage of an impulse and reached a maximum in about one second. The degree of block then gradually decreased. Transmission did not return to the control level until ten or more seconds after the conditioning nerve stimulus. No such block was demonstrated in normal subjects but was reproduced in the partially curarized normal subject.

When trains of stimuli were delivered to the ulnar nerve at various frequencies, there was a characteristic time course of increase in degree of block, as evidenced by the size of the action potential response to the successive stimuli. (Fig. 3.) At first there was an abrupt increase in block followed by a period of decreased block. This was in turn followed by a slow exponential increase in block. A similar sequence of events was demonstrated in partially curarized normal subjects. In contrast, in normal subjects the amplitude of the action potentials was maintained in response to stimulation at rates up to 25 per second.

Neuromuscular transmission during the period

following a train of repetitive stimuli was studied in the same manner described for the period following a single stimulus. A conditioning train was delivered. At variable time intervals after the end of the train a single test stimulus was applied. The muscle action potential in response to the single test stimulus was compared with the response to a single stimulus delivered before the tetanic stimulation. The response to the first stimulus of the conditioning train of stimuli was used for this pre-tetanic control. Again the time course of this post-tetanic change in degree of block was obtained by varying the interval between the end of the tetanus and the test response. (Fig. 4.) After one second there was a period of facilitation of neuromuscular transmission during which the test response exceeded the control response. This post-tetanic facilitation (or decrease in degree of block) disappeared after approximately 10 seconds. Increases in either duration or frequency of the conditioning train increased the degree of facilitation. With a conditioning train frequency of 100 per second there may be discernible action potential responses to only the first 20 to 30 stimuli due to the rapid and marked increase in degree of block at such high frequencies. Nevertheless further increases in train length or frequency were capable of increasing the degree of post-tetanic facilitation. This fact indicates that postjunctional events, initiation and propagation of the muscle action potential and muscular contraction, were not essential to the production of post-tetanic facilitation. Post-tetanic facilitation of neuromuscular transmission could not be demonstrated in normal subjects, nor would facilitation be expected since neuromuscular transmission is already maximal. However, post-tetanic facilitation (or decurarization) could be seen in the partially curarized normal subject. (Fig. 4.)

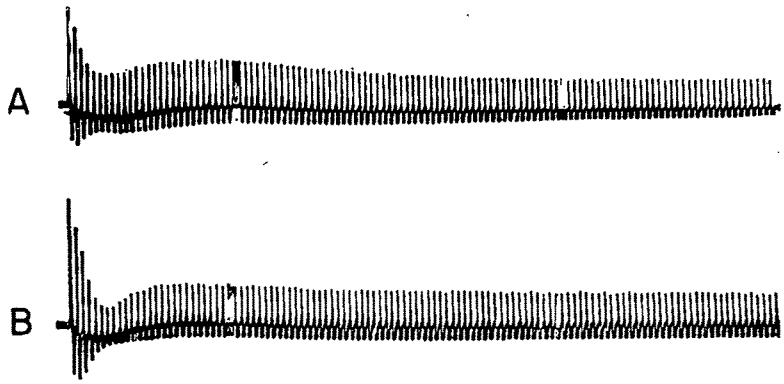


FIG. 5. In a patient with generalized myasthenia gravis a train of 121 stimuli at fifty per second was followed after a five second pause by a similar train of stimuli. A, the response to the first train showing the typical decreased muscle action potential size; B, the response to the second train showing post-tetanic facilitation of the first action potential followed by more rapid decline of subsequent potentials.

In order to investigate this myasthenic post-tetanic facilitation further the conditioning train of stimuli was followed at different time intervals by a test train of stimuli. Again the first action potential response to the test train exhibited post-tetanic facilitation but subsequent responses declined more rapidly. (Fig. 5.) Thus the post-tetanic facilitation of transmission of a single impulse appeared to be at the expense of the ability to transmit subsequent impulses.

SUMMARY

The partial block of transmission at the neuromuscular junction in generalized myasthenia gravis manifests several characteristic features.

1. There is a slight degree of block to the passage of a single impulse.

2. Following the passage of a single impulse there is an increase in the degree of block which reaches a maximum about one second after the passage of the impulse. Ten seconds may be required before the degree of block decreases to its initial level.

3. The application of a train of impulses to the neuromuscular junction results first in a progressive increase in block to the passage of the impulses. This is followed by a transient decrease in block which in turn is followed by a progressive increase in degree of block. The

magnitude of this increase in block increases as the stimulating frequency is raised.

4. Repetitive nerve stimulation is followed after a brief interval by a period of facilitation of neuromuscular transmission. This post-tetanic facilitation represents a decrease in the myasthenic block and is probably prejunctional in origin. The degree of post-tetanic facilitation is increased by increasing the duration or frequency of the tetanic stimulation. The post-tetanic facilitation of transmission of a single impulse is at the expense of the myasthenic subject's ability to transmit subsequent impulses.

While such a study provides no direct evidence regarding the cause of the myasthenic block, it does afford a detailed description of this block. Such a description provides a basis upon which comparisons can be made between the myasthenic block and pharmacologically produced blocks; it gives a frame of reference against which drug-produced changes in the myasthenic block may be measured. The close resemblance between the neuromuscular block in myasthenia gravis and the block produced by *d*-tubocurarine in normal subjects has been pointed out. This indicates that the myasthenic block could be produced by a competitive (or curare-like) block.

Alterations in Neuromuscular Transmission in Myasthenia Gravis As Determined by Studies of Drug Action*

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It is now generally accepted that neuromuscular transmission is normally mediated by acetylcholine (ACh), which is released from the motor nerve ending following stimulation of the nerve. ACh depolarizes the muscle endplate, initiating the endplate potential, which in turn initiates the propagated action potential and contraction of the muscle fiber. The ACh is normally quickly hydrolyzed into acetate and choline by cholinesterase concentrated in the region of the endplate, following which repolarization occurs and the endplate is thereby made ready to be acted upon again.

Impairment of transmission at the neuromuscular junction, i.e., neuromuscular block, may result from either excessive or deficient action of ACh on the endplate, or from drugs which simulate these effects. The administration of ACh in excessive concentration, or of an anticholinesterase compound such as neostigmine which permits the local accumulation of an excessive concentration of endogenous ACh, results in abnormally prolonged depolarization of the endplate. While it is depolarized the endplate cannot be stimulated, and neuromuscular block ensues until the ACh is hydrolyzed. This "depolarization" type of block, which also occurs in most animal species following the administration of decamethonium or choline, is intensified by the administration of ACh or neostigmine. A second type of block is the "competitive" block. This results following the administration of *d*-tubocurarine which blocks the access to the endplate of ACh released from the motor nerve ending, probably by competing with ACh for a common receptor site in the endplate. The competitive type of block inhibits the depolarizing action of ACh more completely than does the depolarizing type of block. The competitive type of block is reversed following the administration of ACh or neostigmine, which permits the local accumulation of sufficient ACh to displace *d*-tubocurarine and overcome the block.

In an effort to obtain further information on the characteristics of neuromuscular transmission in normal subjects and in patients with myasthenia gravis, a study was made of the effect of ACh on neuromuscular transmission. This was done by injecting ACh into the brachial artery through an indwelling cannula during intermittent supramaximal stimulation of the ulnar nerve and observing the effect on the amplitude of the induced muscle action potentials recorded from the skin over the abductor of the fifth finger. Trains of four supramaximal stimuli were delivered to the ulnar nerve at intervals of 40 milliseconds every two to five seconds and injections made after the fourth train. Stimulation was continued at two- to five-second intervals for several minutes, and then at longer intervals for periods up to several hours. Observations were carried out in twenty-five normal subjects and fifteen patients with generalized myasthenia gravis.

In each of the normal subjects ACh, in doses of 1 mg. or more, produced a brief burst of potentials, accompanied by involuntary movement, lasting five to ten seconds. Immediately afterward there occurred a period of "prompt" depression of induced potentials, lasting about twenty seconds, with even depression of successive potentials. (Figs. 1 and 2.) This was followed by temporary recovery and then by a more prolonged "late" depression of induced potentials which began one to three minutes

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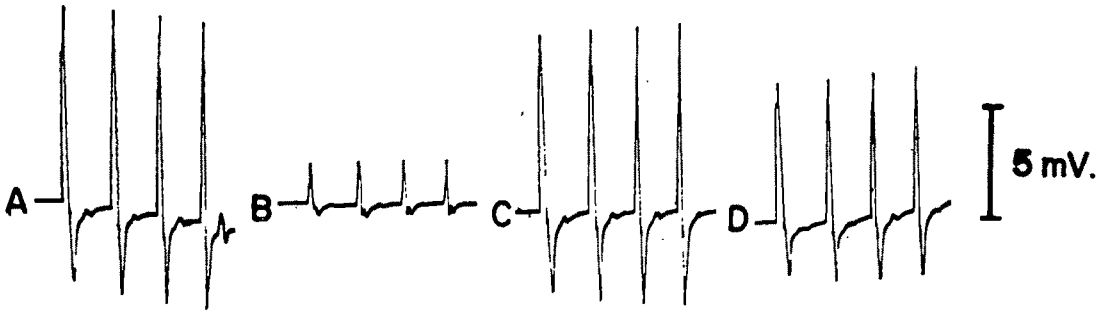


FIG. 1. Prompt and late depressant effects of ACh on the muscle action potentials evoked by nerve stimulation in a normal subject. A, control muscle potentials in response to four supramaximal nerve stimuli at forty-millisecond intervals. B, prompt depression eight seconds after the intra-arterial injection of 5 mg. ACh. C, recovery fifteen seconds after injection. D, late depression twenty-three minutes after injection.

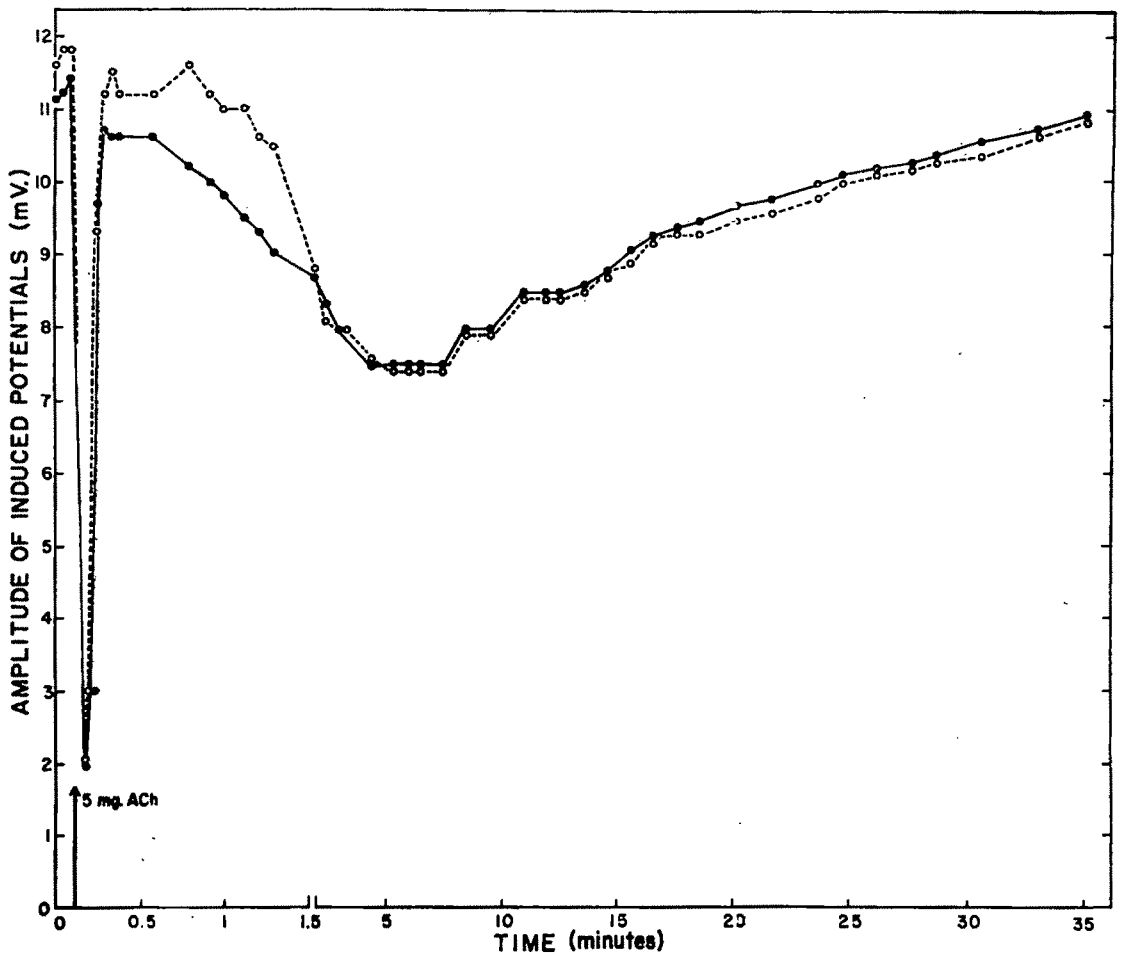


FIG. 2. Time course of the prompt and late depressant effects of intra-arterially injected ACh on the muscle action potential responses to nerve stimulation in a normal subject. The nerve was stimulated by trains of four supramaximal stimuli forty milliseconds apart at two-second intervals. The muscle action potentials in response to the first (●——●) and fourth (o----o) nerve stimuli of each train have been plotted.

after the injection of ACh and lasted for one-half to one hour. Successive potentials were evenly depressed.

The properties of the prompt and late depressions (i.e., neuromuscular blocks) produced by ACh were investigated by observing the effect of sodium acetate, in doses up to 200 mg., had no effect on induced potentials. The intra-arterial injection of choline chloride in doses of 5 to 30 mg. produced prolonged depression of induced potentials which resembled the late depression produced by ACh. Successive potentials were evenly depressed. The depression produced by these doses of choline was enhanced by the injection of ACh or neostigmine (Fig. 5), indicating that it too is a depolarizing type of block. Larger doses of choline produced more marked depression than occurred (late) following ACh. This was enhanced by the injection of ACh, but reversed to a slight extent by small doses of neostigmine, manifesting properties of a mixed type of block. The depression produced by choline differed from the late depressant effect of ACh in its more rapid onset (ten seconds after injection), but otherwise the time course was the same. It seems likely that the late depression produced by ACh is due to choline liberated following the hydrolysis of ACh, and that the longer latent period represents the time required for hydrolysis to occur. The main difference between the depressant effect of choline and of

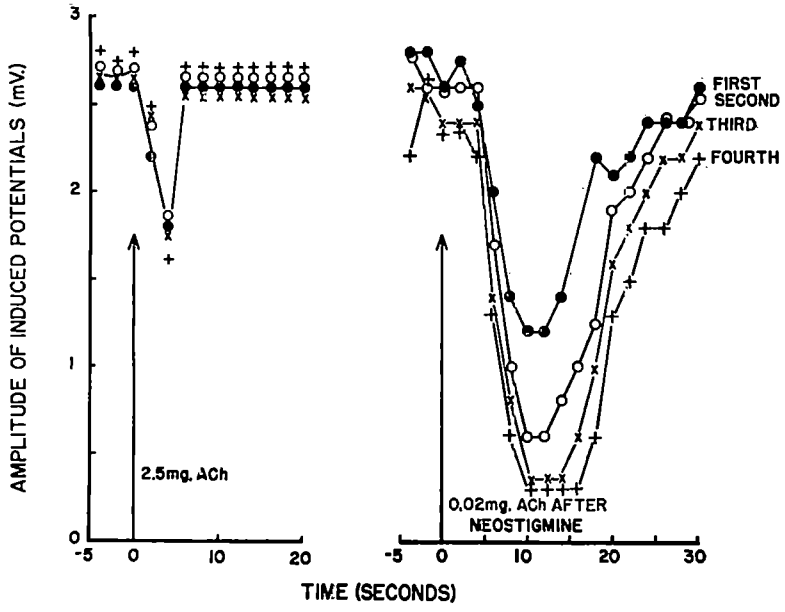


FIG. 3. Potentiation and prolongation by neostigmine of the prompt depressant action of intra-arterial ACh on muscle action potentials induced by nerve stimulation in a normal subject. The nerve was stimulated by trains of four supramaximal stimuli forty milliseconds apart at two-second intervals. On the left is shown the prompt depressant effect of 2.5 mg. ACh prior to neostigmine, and on the right of 0.02 mg. ACh administered twelve minutes after the intra-arterial injection of 1.2 mg. neostigmine. Prior to neostigmine, 0.02 mg. ACh had no effect on the induced potentials.

the intra-arterial injection of ACh and neostigmine on each block. The prompt depression was enhanced and prolonged by the prior injection of neostigmine (Fig. 3), indicating that it has the properties of a depolarizing block. The late depression was also increased following the administration of ACh or neostigmine (Fig. 4), indicating that this too has the properties of a depolarizing type of block. The time course of the prompt depression produced by ACh is compatible with this being a direct effect of ACh on the motor endplates, since this compound is rapidly hydrolyzed. Since the late depression did not begin until one to three minutes after the injection of ACh and since it persisted for one-half to one hour, long after hydrolysis of the ACh had occurred, the possibility that it might be due to one of the products of hydrolysis of ACh was investigated. The intra-arterial injection of

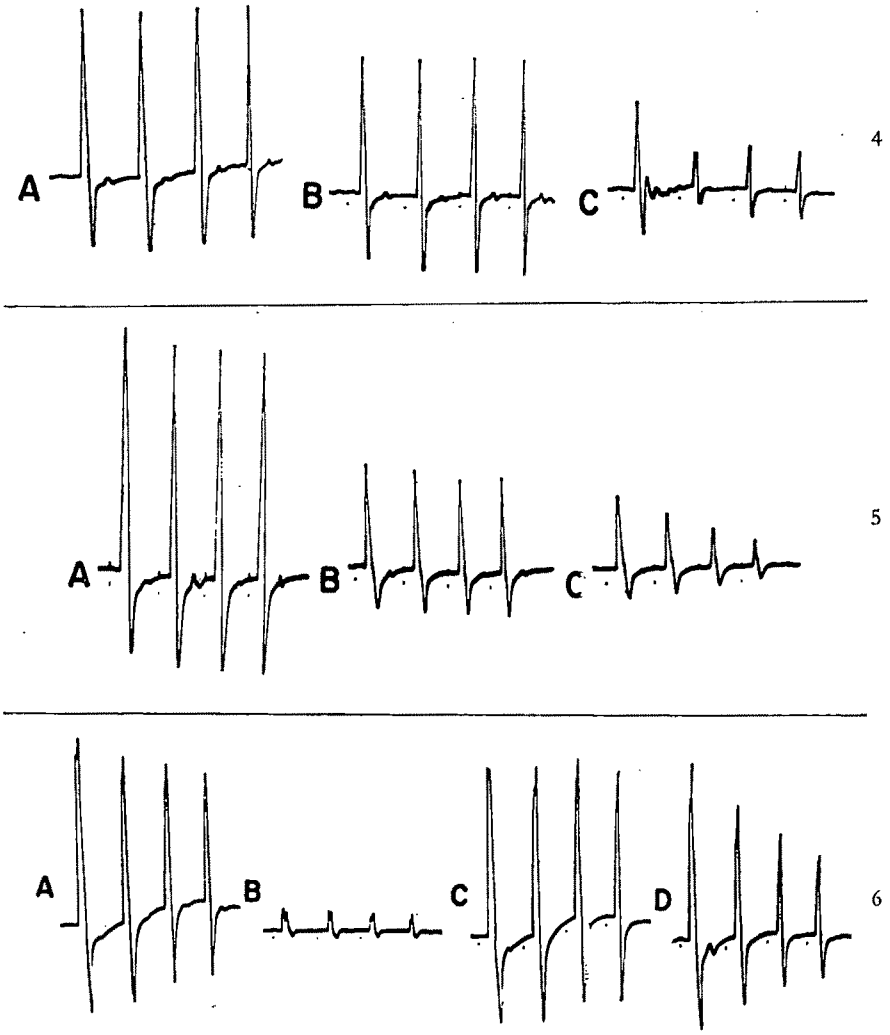


FIG. 4. Depressant effect on induced muscle action potentials of neostigmine administered following their (late) depression by ACh in a normal subject. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, late depression following the intra-arterial injection of four doses of 10 mg. ACh. C, further progressive depression and repetitive firing after the initial potential, thirty-five seconds after the intra-arterial injection of 0.5 mg. neostigmine.

FIG. 5. Depressant effect on induced muscle action potentials of neostigmine administered following their depression by choline in a normal subject. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, depression produced by the intra-arterial injection of 30 mg. choline. C, further progressive depression seventy-five seconds after the intra-arterial injection of 1 mg. neostigmine.

FIG. 6. Prompt and late depressant and reparative effects of ACh on the muscle action potential responses to nerve stimulation in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, prompt depression ten seconds after the intra-arterial injection of 5 mg. ACh. C, repair of the myasthenic defect twenty-two seconds after injection. D, late depression seven minutes after injection.

ACh was in the dose-effect relationship, similar degrees of depression being produced by choline in doses three times that of ACh. Since each milligram of ACh yields 0.8 mg. of choline on hydrolysis, one might expect the reverse to occur. However, it is also possible that ACh these potentials was more marked. As in the normal subjects the prompt depression was enhanced by the prior injection of neostigmine, indicating that it is a depolarizing type of block. On the other hand, the late depression produced by ACh was reversed by the administration of

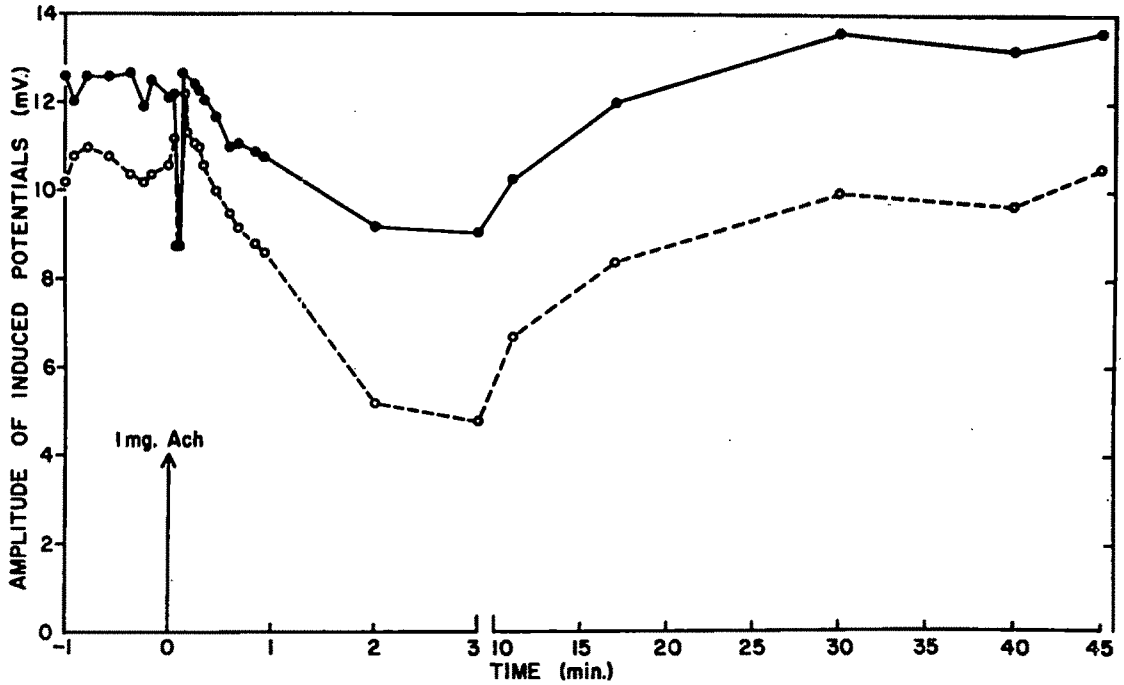


FIG. 7. Time course of the prompt and late depressant and reparative effects of intra-arterially injected ACh on the muscle action potential responses to nerve stimulation in a myasthenic patient. The nerve was stimulated by trains of four supramaximal stimuli at five-second intervals. The muscle action potentials in response to the first (●—●) and fourth (o----o) nerve stimuli of each train have been plotted.

hydrolyzed at the neuromuscular junction may give rise to choline in more intimate contact with the endplates than would result from the injection of choline.

In each of the patients with myasthenia gravis the injection of ACh, in doses of 1 mg. or more, produced transient stimulation of motor unit activity and involuntary movement, followed by prompt and late depressions of induced potentials having the same time course as in normal subjects. (Figs. 6 and 7.) In addition there was transient increase in amplitude, or repair, of the latter potentials which had been initially reduced in amplitude, manifesting the decrement characteristic of myasthenia gravis. This repair occurred between the prompt and late depressions, and lasted for two minutes. The degree of prompt depression of the latter potentials of a train was less in myasthenic patients than in normal subjects, while the late depression of ACh or neostigmine (Fig. 8), indicating that it is a competitive type of block in the myasthenic patients, in contrast to the depolarizing type of block which occurred in normal subjects. The time course of the late depression produced by ACh again suggested that this depression might be due to one of the products of hydrolysis of ACh. Once more sodium acetate had no effect but the intra-arterial injection of choline chloride, in doses of 5 to 30 mg., produced transient increase in the induced potentials followed by prolonged depression which resembled the late depressant effect of ACh. The depression produced by choline, which was more marked in the later potentials of a train in myasthenic than in normal subjects, was promptly reversed by ACh or neostigmine (Fig. 9), indicating that it is a competitive type of block in myasthenic patients. The late depression produced by ACh would therefore appear to be due to choline

released following the hydrolysis of ACh. As in the normal subjects, approximately three times as much choline as ACh was required to produce comparable degrees of depression, again suggesting that choline derived from ACh hydrolyzed at the neuromuscular junction may have more ready access to the endplate than injected choline.

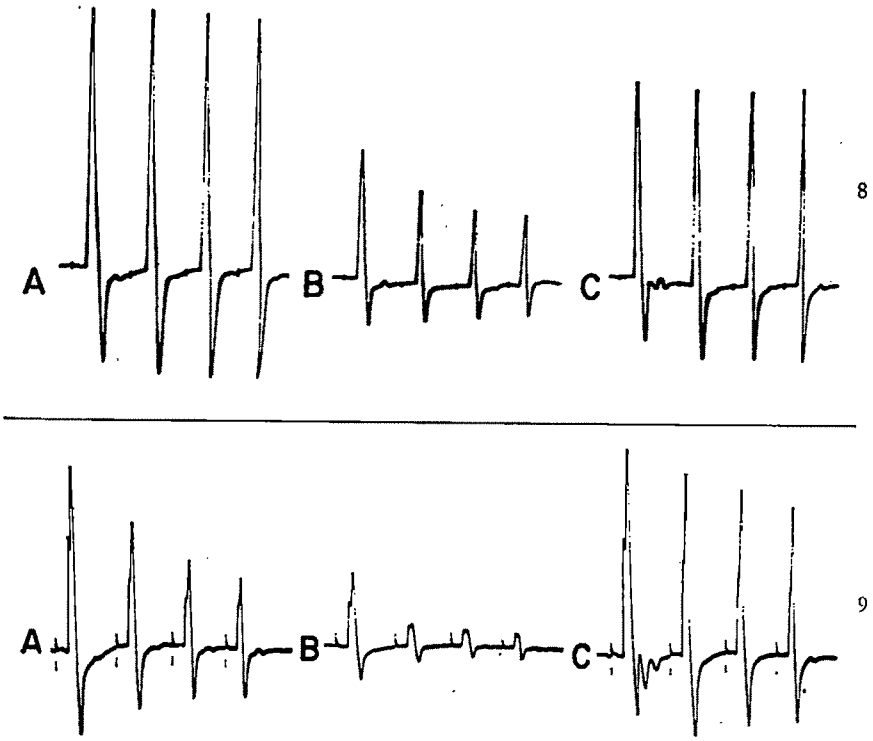


FIG. 8. Reversal by neostigmine of the late depression of induced muscle action potentials produced by ACh in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, late depression following the intra-arterial injection of five doses of 7.5 mg. ACh. C, reversal of the depression two minutes after the intra-arterial injection of 1 mg. neostigmine.

FIG. 9. Reversal by neostigmine of the depression of induced muscle action potentials produced by choline in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, depression following the intra-arterial injection of 100 mg. choline. C, reversal of the depression seven minutes after the intra-arterial injection of 1 mg. neostigmine.

It is not known if the observed effects of injected ACh and choline have faithfully reproduced the actions of the endogenous compounds released during neuromuscular transmission. Nevertheless these effects do indicate that the defect in neuromuscular transmission in myasthenia may be due to a competitive block produced by choline released in a normal manner during neuromuscular transmission following hydrolysis of endogenous ACh. The competitive type of block inhibits the depolarizing action of ACh more completely than the depolarizing type of block, and inhibition of the depolarizing action of endogenous ACh released following nerve stimulation would explain the neuromuscular block of myasthenia gravis. It is not yet known why choline produces a competitive type of block in myasthenic patients and a predominantly depolarizing block in normal subjects. Studies are in progress to determine whether this is due to an abnormal response of the myasthenic endplate to choline itself, or to the formation in myasthenic patients of a product of choline which is a competitive blocking agent. Churchill-Davidson and Richardson have found a similar abnormal response of myasthenic patients to decamethonium, and we have likewise observed that decamethonium produces a competitive type of block in these patients, in contrast to a predominantly depolarizing block

in normal subjects. This suggests that the myasthenic endplate may react abnormally to a variety of compounds which normally produce depolarization, the response to choline being of particular importance because of the release of this compound following motor nerve activity, ACh release and hydrolysis.

SUMMARY

The injection of ACh was found to produce, in normal subjects and in patients with myasthenia gravis, transient stimulation of motor unit activity followed by prompt transient depression of induced potentials, attributable to the depolarizing action of ACh. In myasthenic patients this was followed by a period of transient potentiation of induced potentials. In both normal subjects and myasthenic patients there then ensued a late prolonged depression of induced potentials, attributable to choline released as a result of hydrolysis of ACh. This late depression has the properties of a depolarizing block in normal subjects and a competitive block in patients with myasthenia gravis. These observations indicate that the defect in neuromuscular transmission in myasthenia gravis may be due to a competitive (ACh inhibitory) block produced by choline released in a normal manner during neuromuscular transmission following hydrolysis of endogenous ACh.

Neuromuscular Transmission in Myasthenia Gravis*

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It is now generally accepted that the functional abnormality in myasthenia gravis is a failure of neuromuscular transmission. Previous theories as to the causation of this disease have been based either on the unproved existence of a curare-like substance or on the contradictory results of studies of the action of acetylcholine in normal and myasthenic subjects. The introduction of the decamethonium salts (C 10), which act in a similar manner to acetylcholine,[1,5] offered a unique opportunity for re-investigation of this controversial matter.

In our studies we used a combination of volitional and electromyographic technics following intravenous injection of decamethonium iodide in both normal and myasthenic subjects.[2] The response of the various muscle groups throughout the body could thus be measured.

In normal subjects a dose of 2.5 mg. of decamethonium produced a 70 to 80 per cent reduction in the height of the action potential of the hypothenar muscles on supramaximal stimulation of the ulnar nerve and resulted in some muscle weakness. A subsequent intravenous injection of an anticholinesterase compound, tensilon,® at the height of the decamethonium paresis led to a profound increase in the degree of generalised weakness. In other words, injection of a neostigmine analogue in the presence of decamethonium paresis leads to potentiation of the depolarisation block.

Localised Myasthenia Gravis. In myasthenic subjects a completely different type of response was encountered. In those patients in whom the disease was localised to a few muscle groups (localised myasthenia gravis) and particularly in those in whom the symptoms of the disease were minimal, large doses of decamethonium could be tolerated without any evidence of a reduction in the height of the action potential or in volitional activity. For example, in a twenty-one year old woman, the injection of 10 mg. of C 10 failed to produce any detectable signs of paralysis, yet as little as 3 mg. is sufficient to produce extreme weakness in normal subjects under similar conditions. Furthermore, over 60 per cent of the dose injected into this myasthenic patient could be recovered from the urine and could be shown to possess potent depolarising activity when injected into the neck vein of the chick.[4] This resistance to the depolarising action of decamethonium was found to be present both before and six months after surgical removal of the thymus. Patients showing such marked tolerance to decamethonium are rare; there is little doubt that if further doses were given the patient would ultimately show signs of muscular weakness.

Generalised Myasthenia Gravis. In those patients in whom the myasthenic weakness was generalised (generalised myasthenia gravis) this apparent resistance to the depolarising action of C 10 previously described in the localised type was much less marked. The first muscles to show signs of paralysis due to the C 10 were always those which were clinically weak. When decamethonium finally brought about a neuromuscular block in the muscle of the myasthenic subject this block was not of the depolarising type but was a non-depolarisation (competitive inhibition) block. Evidence for this was that the block could readily be reversed by neostigmine or tensilon, was greater for tetanus than for twitch and also was unaccompanied by muscle tightness and fasciculations seen prior to depolarisation block. Nevertheless the brief improvement in muscle strength which was seen electromyographically in some myasthenic subjects was taken as evidence of depolarisation preceding onset of non-depolarisation block.

COMMENTS

These observations on patients with myasthenia gravis after injection of decamethonium

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suggest that the abnormality is one of an alteration in the response of the motor endplates, namely, a dual response (that is, depolarisation followed by non-depolarisation block).[3,4] Somewhere in the transition from the pure depolarisation response of the normal subject to that of the dual response which is best seen in the clinically weak muscles of myasthenic subjects there must be an intermediate position in which the endplates are resistant to depolarisation. The non-weak muscles of myasthenic subjects show this type of response which is best seen, therefore, in cases of localised myasthenia gravis.

The fact that these patients only have a few muscles showing clinical weakness, yet are able to tolerate large doses of intravenous C 10 without paresis, suggests that in myasthenia gravis the alteration in the response of the motor endplate resists depolarisation and, finally, the threshold for excitation is raised so that a non-depolarisation block ensues. Clinically the presence of resistance to the depolarising activity of C 10 can be used as a test for the presence of myasthenia; this has been found particularly useful in differentiating hysterical paresis from that due to localised myasthenia.

It is important to stress that the clinically weak muscles of the myasthenic are the first to be affected by decamethonium. If the myasthenic weakness was due either to a circulating or locally produced curare-like substance, then one would expect these muscles to be the most resistant to the depolarising activity of decamethonium because the two types of block are antagonistic. Such is not the case. Our findings with decamethonium are inconsistent with such a hypothesis and can best be interpreted on the basis of the dual response.

A dual mode of response to decamethonium identical to that observed by the authors in myasthenia gravis has been described as occurring in various species of animals.[5] Thus while cat, avian and frog muscle respond to decamethonium by pure depolarisation, the muscles of monkeys, dogs and hares can exhibit features of both depolarisation and non-depolarisation block with this substance.

Although there is no direct evidence as yet that the motor endplates in man respond to acetylcholine or one of its breakdown products in this dual manner, such a conception would account for all the clinical features of myasthenia gravis.

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Comparison of Simultaneously Recorded Electrical and Mechanical Activity in Myasthenia Gravis Patients and in Partially Curarized Normal Humans*

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OBJECTIVE testing of muscle function has been performed in myasthenia gravis patients by means of mechanograms and electromyograms.[1–4] To date none of these studies has utilized simultaneous recording of tension and action potentials although discrepancies between electrical and mechanical activity were pointed out by Harvey and Masland in 1941.[1] In myasthenia gravis patients these workers were unable to demonstrate any alteration from normal in the action potential of a single maximal induced twitch although they did find a progressive decline in amplitude of successive action potentials with repetitive stimulation at rates below 40/sec. On the other hand, Lindsley[2] found that the tension developed by single twitches in myasthenics was less than normal and this single twitch tension increased with the administration of neostigmine. Furthermore, Odom, Russel and McEachern,[3] and Pritchard[4] were unable to demonstrate any alteration in tetanus tension in myasthenia gravis patients unless stimulation rates faster than 80/sec. were used. The present paper is an attempt to resolve these views by measurement of simultaneous electrical and mechanical activity of skeletal muscle in myasthenia gravis patients and in partially curarized, otherwise normal, subjects. The results indicate the following: (1) tension studies are more accurate indices to the degree of muscle weakness than the electromyogram and (2) there may be changes in the contractile ability of the muscle in these two conditions at a time when no evidence of neuromuscular transmission block can be observed.

Apparatus and Method. These have been described in detail in a previous paper.[5] Figure 1 is a diagram of the apparatus we used. After suitable splinting, the thumb was placed in a

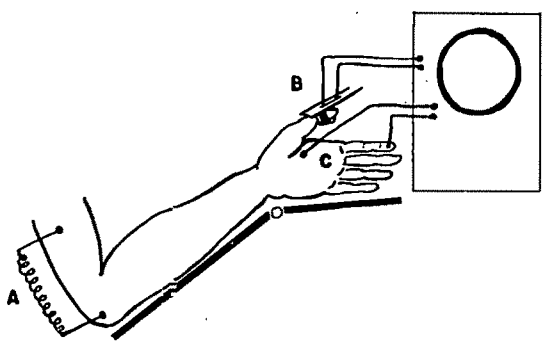


FIG. 1. Apparatus: A, stimulator; B, strain gauge to measure tension; C, electrodes to pick up action potentials. Both A and B are recorded on dual-beam cathode ray oscilloscope, the screen of which is photographed on 35 mm. film moving continuously at the rate of 1,200 in./min.

stirrup which was attached to an overhanging metal bar to which strain gauges were bonded. When the thumb was adducted, the bar was bent very slightly and the strain gauge output increased, causing a deflection of one beam of a double-beam cathode ray oscilloscope. The other beam of the oscilloscope was deflected by action potentials picked up from a surface electrode on the belly of the adductor pollicis

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muscle. The short adductor of the thumb was induced to contract by supramaximal percutaneous stimulation of the ulnar nerve at the elbow.

RESULTS

Figure 2 shows the results with 3/sec. stimulation. No fusion of successive single twitch tensions occurred at this stimulation rate either in the myasthenia gravis patients or in the normal subjects. Therefore, with 3/sec. stimulation one must note: (1) the initial single twitch and (2) the pattern of the successive single twitches. There was no difference between the tension and the action potential amplitude of the initial single twitches of normal subjects and myasthenia gravis patients. No significance can be placed on the finding that the mean initial single twitch tension was somewhat less in the myasthenics because no attention was paid to initial muscle length from individual to individual and the groups were not homogeneous as to age and sex. However, while the tension of successive twitches did not decrease in all the normal subjects, it did decrease significantly in all the myasthenics so that the tension developed by the fifth twitch was less than that developed by the first in all the myasthenics. There was a discrepancy between the tension and the electromyogram because the decrease in tension of successive twitches at 3/sec. was accompanied by a decrease in action potential amplitude in only two of the four myasthenia gravis patients.

In the three normal subjects after the intravenous administration of *d*-tubocurare there was a decrease in the initial single twitch tension with a further decrease in the tension of successive twitches at 3/sec. In this instance there again was apparently no correlation between the electrical and mechanical activity of the muscle since in only one of the three subjects was there a decrease in the amplitude of the successive potentials.

After the administration of neostigmine there was a slight but significant increase in initial single twitch tension in two of the four myasthenia gravis patients, and in all four patients the decrement in successive twitch tensions was less marked than it had been before the drug was given. Again there was no correlation between electrical and mechanical activity since none of the myasthenics showed an increase in initial action potential amplitude and in only one of the four was there less marked decrement in the amplitude of successive action potentials after neostigmine.

Figure 3 shows the results with 30/sec. stimulation for one second. Complete tetanus occurred at this stimulation rate. Tension within the normal range developed in all four myasthenics

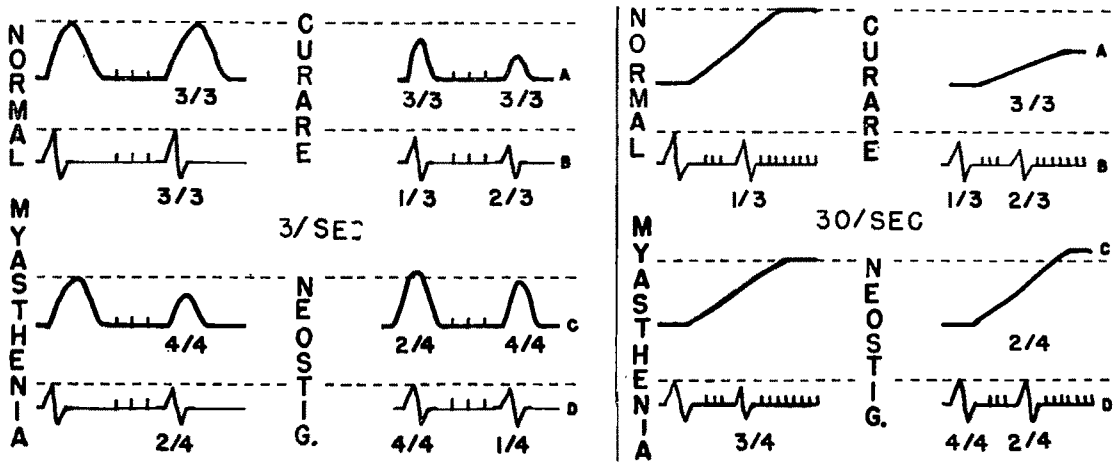


FIG. 2. Three per second stimulation: A and C, tension-time curves of first and fifth twitches; B and D, simultaneously recorded action potentials. Time relationships of tension-time curves and action potentials are not accurate in this and subsequent figures. In this and subsequent figures, the ratio equals number of subjects showing a given pattern/total number of subjects.

FIG. 3. Thirty per second stimulation: A and C, tension curves of beginning of tetanus to plateau; B and D, simultaneous first and fifth action potentials.

and three of the four showed a significant decrease in the amplitude of successive potentials. (However, one of the normal subjects showed a similar electromyographic pattern.) After neostigmine two of the three myasthenics who had had an abnormal electromyogram before the drug showed a significant increase in developed tension. In these two patients the successive action potentials were sustained better than they had been before neostigmine. After neostigmine there was no significant change in tension in the other patient who had had an altered electromyogram before the drug even though this pattern was restored toward normal.

After intravenous *d*-tubocurare less tension with 30/sec. stimulation developed in all three normal subjects. On the other hand, only one of the three showed a decrease in initial potential amplitude and only two of the three showed a decrease in amplitude of successive potentials, which was not present prior to the administration of the drug.*

To recapitulate, in myasthenia gravis patients and in partially curarized otherwise normal subjects mechanical activity can be altered without a corresponding change in electrical activity. This probably means that alterations can occur in the contractile processes of muscle in these two conditions apart from any change in neuromuscular transmission. It is apparent from the results of this study that changes in tension are more accurate measures of muscle weakness than are changes in the action potential. The method of recording simultaneous mechanical and electrical activity with indirect supramaximal stimulation of the muscle appears to be an excellent objective measure of the effects of muscle relaxant drugs in man. This method eliminates many of the objections which have been raised to dynamometer methods of testing muscle strength.[6] If a choice between mechanical and electrical activity must be made, we believe that the recording of mechanical activity would be more useful, more accurate, more convenient and less expensive.

Rather than speculate at this point upon why a difference should exist between electrical and mechanical activity, we would like to present some effects of tetanus upon the single twitch in myasthenia gravis patients and in partially curarized, otherwise normal individuals. (Fig. 4.) It can be seen that in normal subjects a single twitch following a tetanus of 30/sec. for one second develops greater tension than a pretetanus single twitch. This normal post-tetanic potentiation of twitch tension is unaccompanied by any change in the action potential and lasts

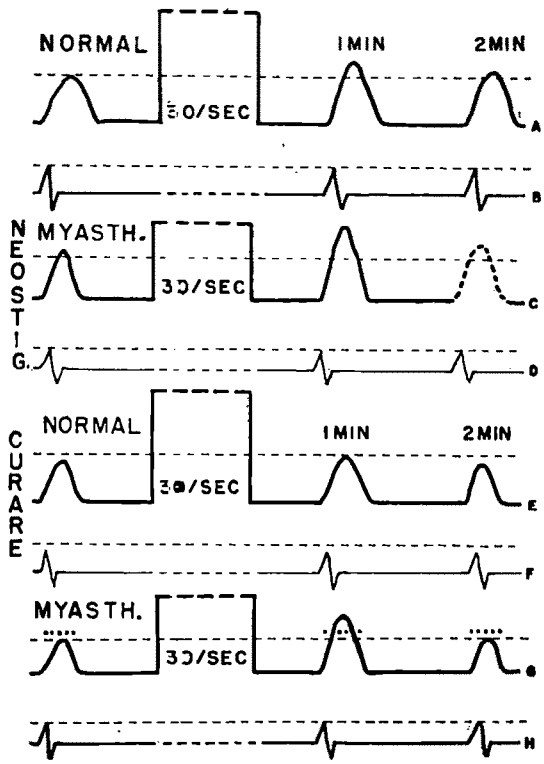


FIG. 4. Effects of tetanus on single twitch: A, C, E and G, tension-time curves of single twitch, tetanus of 30/sec. for one second, and single twitch at one and two minutes post-tetanus; B, D, F and H, simultaneous action potentials of single twitches. Dotted lines in G indicate the tension developed by the pretetanus twitch after neostigmine.

at least ten minutes.[5] In the myasthenia gravis patients and in the partially curarized normal individuals we found that post-tetanic potentiation of twitch tension did occur but did not last as long as it did in normal uncurarized subjects. We had expected to find that the post-tetanic potentiation of twitch tension in myasthenia gravis patients and partially curarized individuals would be accompanied by an increase in the dimensions of the action potential (amplitude, duration and area), indicating that more fibers were active as a result of the restoration of neuromuscular transmission by the tetanus.

* It is of interest to note that the individual who showed a decrease in tension but not in electrical activity at both 3/sec. and 30/sec. stimulation after curare had marked symptoms of weakness of laryngeal and pharyngeal muscles.

However, this was not the case since the post-tetanic potentiation of twitch tension was not accompanied by changes in the action potential dimensions. These findings suggest that in myasthenia gravis patients and in partially curarized normal subjects an alteration in the contractile ability of skeletal muscle can account for muscle weakness at a time when there is no alteration in neuromuscular transmission. We do not mean to imply that the myasthenic is identical to the curarized subject, for further analysis of the post-tetanus state shows that this is not so. These data will be reported elsewhere.

When neostigmine was administered to the myasthenia gravis patients we found that post-tetanic potentiation of twitch tension occurred, lasted as long as in normal subjects and was still unaccompanied by changes in the action potential. We interpret these findings to mean that neostigmine can restore muscle strength in myasthenia gravis patients by altering the contractile processes without causing any observable change in neuromuscular transmission.

Post-tetanic potentiation of twitch tension may in part offer an explanation for the fact that at 30/sec. stimulation some myasthenics show normal developed tension despite a progressive decline in action potential amplitude. It is possible that during the course of the tetanus some muscle fibers may alternate between activity and inactivity. When they are in the inactive phase following activity, they are in the post-tetanus state. Then, when they again become active they contribute a greater increment of tension than they would have had they continued to be active throughout the tetanus. This view has been suggested by Brown and Burns[7] to explain similar findings in fatigued cat skeletal muscle.

SUMMARY

In summary, the present study of simultaneous electrical and mechanical activity in myasthenia gravis patients and in partially curarized normal subjects indicates that tension changes at slow (3/sec.) and moderate (30/sec.) stimulation rates are more accurate indices to muscle weakness than changes in the action potential. In addition, the results in these two groups of subjects suggest that the weakness may, at least in part, be due to changes in the contractile ability of the muscle apart from any change in neuromuscular transmission. Neostigmine apparently is capable of restoring the contractile ability of muscle at a time when no alteration in neuromuscular transmission can be observed.

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The Thymus and Myasthenia Gravis*

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THE notion is not a new one that the thymus might have something to do with myasthenia gravis; this is obvious from publications on the subject since the beginning of this century. Furthermore, it is clear that interest in the experimental investigation of the subject has arisen and has been maintained as a result of consistent clinical observations on patients with myasthenia gravis. The idea of trying to produce a myasthenic animal or at any rate of attempting to reproduce the essential features of this condition in the whole or part of an animal has occurred to many, but the results of such attempts as have been published are conflicting and in general have provided little evidence that the thymus influences muscular function. I shall not comment on the results of implanting pieces of thymus gland other than to draw attention to the fact that the duration of effect was transient, and that in no case has the tissue been transplanted to ensure vascular anastomosis with the recipient.

I would like briefly to summarise the published evidence regarding the effects of thymus extracts, for statements are often made about findings by one author not being confirmed by another, when in fact the experiments have not been repeated. In most instances calf thymus extracts have been used; a few extracts have also been made with thymus glands removed from patients with myasthenia gravis. The extracts of calf thymus have usually been made with saline solution and tested in doses equivalent to 0.4 to 20 gm. of fresh thymus. The biologic preparations used to assess the activity of the extracts have been of several types; the method has usually been to produce muscular weakness or fatigue in the unanaesthetised animal or in a nerve-muscle preparation by stimulation of the nerve supplying a muscle or group of muscles, and to observe whether the extract prevented, relieved or induced muscular fatigue.

Adler produced in the dog rapid fatigue of the anterior tibialis muscle in response to repeated indirect tetanic stimulation, after intravenous injection of an extract equivalent to 4 gm. of fresh calf thymus. This effect lasted for about twelve hours and was relieved and prevented by neostigmine. When the extract was injected subcutaneously the effect was delayed in onset but lasted for about twenty-four hours. Extracts of calf spleen did not produce these effects. Klose and Vogt used pressed thymus juice in doses equivalent to 10 to 20 gm. of fresh thymus. When this was injected intravenously or subcutaneously into thymectomised puppies it aggravated the signs of fatigue already evident in these animals. Normal dogs were unaffected. Constant and his colleagues observed, in the cat, depression of the muscle twitch in response to stimulation of its nerve after a saline extract equivalent to 2 gm. of dog thymus was injected into the carotid artery.

A number of attempts have been made to extract thymus glands from myasthenic patients and to test them for curare-like activity. The only evidence of a positive nature is that by Constant and his colleagues who observed depression of the muscle twitch after intracarotid injection of a saline extract equivalent to 1 to 2 gm. of fresh thymus gland.

Choice of Solvent, Biologic Test and Standard. The negative results reported in many of these investigations are probably due to the use of relatively insensitive biological tests and to insufficient doses of thymus, for an estimate of the amounts of tubocurarine required to produce an effect on these test objects shows that each dose of thymus would require to contain curare-like activity equivalent to 0.1 to 1 mg. *d*-tubocurarine. This is beyond the usual total activity obtained from one myasthenic thymus gland. McEachern, at least, was aware of some of these difficulties when he briefly reported his results in a masterly review of the subject ten years ago.

Our first extracts were prepared from myasthenic thymus glands and consisted of prelimi-

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nary extraction with acetone followed by saline extraction of the acetone-soluble residue and of the acetone-dried gland. The combined saline extracts were adjusted to the pH of Tyrode solution. The use of acetone in the extraction process was fortuitous, for it was primarily used for the storage of the thymus glands after their removal from patients. In view of the variations in water and fat content of fresh thymus, the activity of all extracts is referred to a weight of acetone-dried thymus, 1 gm. of which is equivalent to 5 to 7 gm. of fresh thymus gland.

For estimating the activity of the extracts we used the isolated rat phrenic nerve diaphragm preparation described by Bülbring (1946), which is more sensitive to tubocurarine than any of the tests previously described. With slight modifications we were able to detect a depression in the muscle twitch response to as little as 2 μg. of tubocurarine. The selection of *d*-tubocurarine as a standard substance with which to compare the action of thymus extracts was based entirely on the convenient assumption that if any effect of thymus extracts was related to the clinical features of myasthenia gravis, it would approximate more closely to the action of this neuromuscular blocking agent than to any other drug known at that time. Subsequent experiments have shown that the actions of thymus extracts differ in a number of ways from those of *d*-tubocurarine.

Preliminary Results. Biologic assay of human thymus glands obtained at operation from myasthenic patients and at autopsy from non-myasthenic infants and adults showed a wide variation in the activity that can be extracted from the thymus. The results show that the most active extracts were of myasthenic glands whose removal produced the greatest therapeutic benefit and also that some of the infant thymus gland extracts were more active than those of normal adults.

It was now obvious that if these results were related to a function of the thymus, the amount of activity secreted daily from the gland must greatly exceed the amount that can be extracted from it, for the most active myasthenic thymus extract had a total activity equivalent to ¼ mg. of *d*-tubocurarine not sufficient to produce any effects in man. Large batches of thymus glands hence would be required for preparation of extracts; since it was unlikely that these would be obtained in sufficient amounts from myasthenic patients, animal thymus glands should be obtained from very young animals and preferably from foetuses. This is a point of some importance, for in later work with calf thymus extracts we were unable to explain some conflicting and variable results until we found that the Ministry of Food officials, who at the time controlled the supply of material from the abattoir, did not distinguish between young calves and bullocks two to three years old.

Later Methods of Extraction. Thymus extracts prepared in this way are unstable in aqueous solution and lose at least 50 per cent of their activity when stored for twenty-four hours in the refrigerator. They are quickly inactivated by boiling in acid or alkaline solution and a solution of the ashed residue is inactive. Attempts were made to prepare a more stable extract by freeze-drying the aqueous solution. The freeze-dried residue is a buff-coloured amorphous powder, readily soluble in water, 1 mg. of which was equivalent to 0.5 gm. of acetone-dried thymus. Ether and petroleum ether were found to remove only inactive fatty material.

Considerable difficulty arose regarding the processing of large batches of freeze-dried material and the original process of acetone and saline extraction was reinvestigated. When fresh thymus was shaken twice with acetone most of the activity was removed by the acetone and our subsequent method of extraction consisted in concentrating the filtered acetone extract under reduced pressure to a small volume. This concentrate when centrifuged separated into three layers, the lowest of which was brown. The brown layer so obtained is a viscous liquid which when concentrated *in vacuo* yields a brown residue which is readily soluble in water and in saline solution, forming an amber-coloured solution. The brown layer appears to be stable when mixed with acetone and kept in the refrigerator, but aqueous solutions of it at room temperature are unstable.

Batches of foetal whale thymus, human infant thymus and calf thymus, human lymph node and human voluntary muscle have been extracted in this way. The yield of brown layer has been greatest from foetal whale thymus and least from calf thymus. No definite brown layer separated from human lymph node and human voluntary muscle.

Mode of Action. A general study of the actions of thymus extracts has not yet been made; attention has been focussed primarily on their effects on neuromuscular transmission and

voluntary muscular movements by comparing the actions of thymus extracts with those of *d*-tubocurarine and decamethonium (C 10).

Frog Rectus. The response of the isolated frog rectus muscle to acetylcholine is decreased in the presence of tubocurarine and increased by C 10. There is a marked resemblance between the effects of myasthenic thymus gland extract and that of C 10, in which small doses potentiate the action of acetylcholine and larger doses produce a prolonged contracture.

In the initial stages of the investigation this result came as a suprise but was always obtained under the same conditions. The possibility was considered that the extract might contain either one substance with two different types of action, or two different substances. To explore this the freeze-dried material was extracted with alcohol; the alcohol-soluble fraction produced an effect similar to that of *d*-tubocurarine but recovery from the former was more delayed than from the latter.

The precise conditions necessary for the separation of this apparent curare-like fraction have not been determined, for although several attempts were made to repeat this separation from extracts of myasthenic thymus gland they have not always been successful. Furthermore, we have so far failed to obtain a similar fraction from extracts of calf thymus. In all cases the alcohol-insoluble fraction has always increased the response to acetylcholine.

Antagonism with Anticholinesterases. It was an attractive proposition to determine the action of thymus extract in the presence of eserine. The curare-like effect on the frog rectus preparation was not reduced when eserine was added to Ringer's solution, nor was the response of the rat diaphragm preparation to the extract altered in the presence of eserine. The effect of myasthenic thymus extract is not reversed, as is typical of tubocurarine. Similar results have been obtained with foetal whale thymus extract on the cat tibialis/sciatic nerve preparation.

Effects on Response to Direct and Indirect Stimulation. Some experiments were planned to study the effect of thymus extract on the response of the isolated rat diaphragm preparation to indirect and to direct stimulation. It was hoped that the results so obtained would determine whether the action resembled that of tubocurarine more closely than that of C 10. This, of course, is not a critical method of distinction.

When more adequate amounts of foetal whale thymus extract became available by the simplified acetone extraction, the effect of brown layer was observed on the cat tibialis preparation stimulated through its nerve. Small doses of the extract produce an increase in twitch height. Larger doses produce a transient contracture followed by a depression in twitch height. These effects closely simulate those of C 10. The response, however, was not entirely typical of a depolarising effect since tetanus was not sustained.

When the chronically denervated cat tibialis muscle is stimulated directly, the response to thymus extract consists of a contracture similar to that observed after an injection of C 10.

Action on Chicks. Another approach was made by injecting brown layer intravenously into young chicks. Buttle and Zaimis have shown that in this way C 10 produces a rigid extension of the limbs and retraction of the head with a typical spastic paralysis; by contrast, after tubocurarine there is flaccid paralysis and the neck is flexed.

Within five seconds of a dose of 5 mg./gm. of body weight of foetal whale thymus extract the chicks show initially extension of the limbs, fluttering of the wings and irregular respiration, with loss of cheeping; this is followed within a minute by a flaccid paralysis of the limbs and flexion of the neck lasting for five to eight minutes, thereafter the cheep is restored and the chick recovers and walks about within ten to fifteen minutes. Larger doses caused rapid respiratory paralysis and the chicks died within forty-five seconds. Extracts of human lymph nodes and of voluntary muscle when similarly injected had no effect.

In view of the limited amounts of foetal thymus extract, only a few observations were made on the mouse. A modification of the righting reflex test (described by Collier and his colleagues in 1949) was used. The mouse is placed on a revolving drum rotating on a horizontal axis. A positive effect is scored if during a three-minute period the animal fails to remain on the drum. After an intravenous injection of tubocurarine (0.1 μg./gm.) the mice fall from the drum in fifteen to forty-five seconds and usually recover within five minutes. Foetal whale thymus extract in doses of 5 mg./gm. intravenously caused the animals to fall off within one and a half to two minutes; they usually recovered in eight to ten minutes. Larger doses

(7.5 to 25 mg./gm.) caused general paralysis of limbs and of respiration.

While these experiments on unanesthetised animals lend support to the observations made on isolated tissue and other nerve muscle preparations, it is clear that the nature of the action produced by thymus extract does not comply with the criteria demanded of a neuromuscular blocking substance of either a competitive or a depolarising type.

Effect of Thymus Extract on Response to Tubocurarine. Since all the evidence so far obtained indicates that the action of the extract is transient, and at most lasts for only about fifteen to twenty minutes, it was concluded that the prospects of maintaining a state of muscular paralysis in animals could probably be brought about only by frequent injections of extract. Until sufficient supplies of thymus extract became available it was decided to determine whether thymus extract modified the response of the nerve-muscle preparation to neuromuscular blocking drugs, as appears to be the case in patients with myasthenia gravis.

In the cat tibialis preparation thymus extract appeared to increase the sensitivity of the preparation to tubocurarine, an effect not observed with lymph gland extract. Observations on the chick showed that when tubocurarine was given within eighteen minutes of an injection of foetal whale thymus extract the dose required to produce typical flaccid paralysis was only one-quarter of that normally required. When the time between the injection of thymus extract and tubocurarine exceeded twenty minutes, increased sensitivity was not observed.

CONCLUSIONS

No far-reaching conclusions can be drawn from these results for the work has only reached a preliminary stage. There is an immediate need to purify the thymus extract and to determine whether the effects which have been observed are due to one or to more than one active substance. At present, work is in progress on this aspect.

It is not possible to state with certainty that these effects are peculiar to thymus gland extracts but they have not been observed with extracts of lymph nodes and of voluntary muscle. The effects described have been most readily obtained with foetal whale thymus gland and myasthenic thymus gland extracts; the effects seen with calf thymus extracts have been more variable and we do not know whether this is due to quantitative or qualitative differences. Within certain limits of doses used in these experiments the action of thymus extract is transient and the nerve-muscle preparation or unanaesthetised animal recovers spontaneously. The effect is not reversed by neostigmine but it is not known what changes, if any, result from repeated administration of the extract.

Thymus extracts do not produce, in the test objects used, all the characteristic features of myasthenia gravis. The facts so far elicited, however, are not inconsistent with the view that the thymus gland may release or be intimately concerned with the release of a substance which affects neuromuscular transmission.

This assumption has the merit of affording an explanation for the remission of symptoms, on the grounds of a temporary cessation or reduction in the amount of substance released. Whatever the nature of its action it appears, up to a point, to be a reversible one, and in these circumstances removal of the thymus effectively controls the condition.

On the other hand, if the substance is released over a prolonged period, as in patients who have had the disease for many years, its action may then result in irreversible changes at or near the neuromuscular junction which are not influenced by removal of the thymus even though the response to neostigmine is unaltered.

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DISCUSSION

DR. J. ALFRED RIDER: The inhibiting effects of extracts of thymus glands on the rat phrenic nerve-diaphragm preparation as reported by Professor Wilson are of great importance and of unusual interest to all who believe that the thymus gland may play an etiologic role in myasthenia gravis. There are two points in his study which should be re-emphasized: First, the degree of activity of the myasthenic gland in these experiments bore no direct relationship to the severity of the myasthenia gravis as judged by the

neostigmine requirement; second, a very definite correlation was shown between the activity of the thymus glands of myasthenic patients and the beneficial results obtained following thymectomy. If this correlation holds up in further experiments it might be taken to indicate that an inhibiting substance is present in the thymus glands and possibly in other organs of the body or in the lymph nodes as well. Thus in cases in which sufficient amounts of the inhibiting substance were being concentrated or produced in the thymus, thymectomy would be warranted. In patients in whom this condition did not prevail the operation would probably be of no benefit. The etiologic and therapeutic implications are therefore important.

The results of my own experience with thymic extracts and serum from patients with myasthenia gravis have not been as definite or as clear-cut as those of Professor Wilson. The first experiments were begun in 1949, in collaboration with Drs. Constant, Porter and Andronis. Tissue extracts were injected into the carotid artery of cats prepared as described by Porter and Wharton.[1] With this method the brain and cord of the cat is pithed, and the animal is maintained by artificial respiration. The tibialis anticus muscle is used to record muscular contractions. The peroneal nerve, with its blood supply intact, is cut and drawn into a glass tube electrode; branches of the peroneal nerve to other muscles are severed. The nerve is stimulated once every three seconds by break shocks from a large inductorium. When the contractions have been regular for several minutes, 5 to 10 cc. of the extract to be tested are injected into the carotid artery. The extracts used were made by emulsifying the organs in a 1 to 5 dilution of normal saline for a period of several minutes and passing the emulsion through a glass-wool filter. The resultant filtrate was employed in all determinations.

In seven experiments using extract of myasthenic thymus removed surgically, diminution in muscular contraction occurred in five instances. Results in two tests were doubtful. In three experiments using extract of myasthenic thymus removed at autopsy, muscular contraction was doubtful in one instance and positive in two. This means that there was decrease in muscular contraction which began in two or three minutes and persisted for several minutes. In four experiments with extracts of non-myasthenic thymus removed at autopsy, diminished muscular contraction occurred in each case. Two experiments using extracts of spleen removed from the same patients increased the height of muscular contractions in each case. In all but one of the experiments in which the muscular contractions were reduced, recovery of the contraction height took place after several minutes. The data from these experiments would seem to indicate that saline extracts of the thymus gland, whether derived from normal subjects or from patients with myasthenia gravis, contain a substance capable of reducing neuromuscular response to a given stimulus.

More recently I performed a different group of experiments in an attempt to demonstrate the inhibiting effects of myasthenia gravis serum, as well as extracts of normal and myasthenic thymus gland, upon a rat nerve-muscle preparation. The technic used was a modification of that described by Ingle.[2] Young adult white rats of the Sprague-Dawley strain, male and female, adrenalectomized and non-adrenalectomized, were used as test animals. These animals were anesthetized with nembutal,® 35 mg./kg. of body weight, given intraperitoneally. The jugular vein was canalized for the injections. The sciatic nerve was stimulated supramaximally with a direct electrical current at the rate of five per second, and the gastrocnemius contractions were recorded upon a kymograph. The serum to be tested was injected, undiluted, in amounts of 0.2 to 1 cc. The thymic extracts were either water or saline homogenates in a dilution of 1 to 5 filtered through a glass filter or alcoholic extracts evaporated to dryness and then redissolved in water or saline so that the final dilution represented a 1 to 5 concentration.

The results of thirty-eight such experiments were as follows: Four experiments using extracts of beef thymus and four experiments using extracts of pork thymus showed no effect upon neuromuscular response. In twelve experiments with extracts of thymus obtained from patients with myasthenia gravis at operation or autopsy a definite decrease in neuromuscular contractions occurred in four animals with no decrease in eight. In twelve experiments using myasthenia gravis serum a definite decrease in muscular contraction was noted in two animals, but in ten there was no decrease in contraction. Extracts of thymus gland removed from newborn infants at autopsy were injected into six animals; a decrease in muscular contraction was noted in two. Numerous injections with normal serum, saline and alcohol had no effect upon muscular contractions.

It must be emphasized that in all of the experiments in which muscular contraction was diminished there was a decrease and not infrequently a temporary complete cessation of respiration. When respiration was resumed there was obvious hyperpnea. Whether this effect resulted from inhibition of the muscles of respiration by the injected substance in a manner analogous to the inhibition produced on the gastrocnemius muscle, or whether this was part of an anaphylactoid reaction could not be determined. If the latter were the case, then the results reported might be

[1] PORTER, E. L. and WHARTON, P. S. Irritability of mammalian nerve following ischemia. *J. Neurophysiol.*, 12: 109–116, 1949.

[2] INGLE, J. D. The quantitative assay of adrenal cortico-hormones by the muscle work test in the adrenalectomized nephrectomized rat. *Endocrinology*, 34: 191–203, 1944.

of much less significance since the observed effects might have been only secondary and fortuitous.

It appears, then, that a substance is present in the serum and in the thymus gland of patients with myasthenia gravis which will decrease the muscular contractions in a mammalian nerve-muscle preparation. This effect, however, cannot be consistently demonstrated. Obviously, experiments of this type are difficult to perform. First, the amount of thymus gland available in any one case is small. Second, the effect of the glandular extract upon muscular contraction probably depends upon the strength or concentration of the inhibiting substance it contains. This, in turn, may or may not be related to the severity of the disease. It is certainly possible that negative results were obtained when a sufficient amount of material was not present in the thymus gland or when the amount of thymic extract injected was too small to induce the response. Then, too, in our experiments no real attempt was made to concentrate the presumptive inhibiting substance. Other tissue extracts and various precipitations should be investigated to see if it would be possible to concentrate this substance. Although these investigations show promise, it is apparent that a great deal of additional work remains to be done.

DR. SUMNER ZACK: Dr. Wilson's pioneering work on thymus extracts from myasthenic thymuses is well known.

We have been testing extracts prepared according to the acetone extraction method of Dr. Wilson (1952) on isolated frog sartorius muscles arranged in a chamber fitted with electrodes which permit direct and indirect stimulation. The isometric mechanogram was recorded with a strain gage. Acetone extracts prepared from thymus glands obtained from fifteen myasthenic patients, three spleens and two samples of striated muscle were tested.

Both acetone-soluble and acetone-insoluble fractions markedly decreased the muscle tension curve in two to twenty minutes. When the muscles became inexcitable on indirect stimulation, they also were inexcitable on direct stimulation. The active material is heat-stable, dialyzable and is antagonized by $CaCl_2$ added to the extracts.

Concentrations of potassium two to forty-four times the potassium concentration of frog Ringer's solution were found in the extracts. These concentrations of potassium are sufficient to produce the muscle inhibition observed.

More refined extraction procedures will be needed to find possible blocking activity in thymus extracts.

Present Status of Thymectomy in Treatment of Myasthenia Gravis*

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FEW medical papers have had the catalytic effect of that published in 1939 by Blalock and associates[1] describing apparent success in the treatment of myasthenia gravis by removal of a tumor from the region of the thymus gland. The paper was most timely and is generally credited with introducing the era of frequent thymectomy which, after a feeble beginning in 1941, became fully established in 1942. The paper was timely because by 1939 neostigmine and sulfonamide therapy had been available for only a short time; furthermore, thoracic surgery only recently had attained relative maturity. As a consequence of these developments a number of well trained and experienced thoracic surgeons were available and anxious to perform thymectomy on patients who had myasthenia gravis, and physicians, particularly those who were serious students of myasthenia gravis, had been sufficiently impressed by the gradual accumulation of evidence since 1901 of a relationship between myasthenia gravis and the thymus gland that they were willing to make available to the thoracic surgeons certain of their patients who had this disease.

Now, more than thirteen years after the era of frequent thymectomy began, the investigators who have attempted to determine the value of thymectomy do not present a united front with regard to its advisability in the treatment of myasthenia gravis. Naturally this failure to agree creates confusion among those who look to these investigators for guidance. The consensus of those students of myasthenia gravis in the United States who have not participated directly in the study of large series of patients undergoing thymectomy apparently was reflected by Westerberg,[2] who stated in effect that thymectomy evidently is not of great value or it would not be so difficult to determine whether or not it is helpful.

We believe that recently available data allow some clarification of the confusion regarding the value of thymectomy and allow for the conclusion that the operation is of value, at least for certain patients who have myasthenia gravis. Consequently we propose to review critically the more significant reports of recent years against the background of earlier contributions, compare them with newly prepared data from a study of our cases and prepare conclusions that will be of practical value in the selection of patients for thymectomy.

The first problem facing those who attempt to assess the value of thymectomy is to determine whether or not this surgical procedure influences beneficially the course of myasthenia gravis. The problem would be simple except for two factors. First, it was apparent early in the studies that not all patients undergoing thymectomy were improved as a result of operation. Second, it was well known that the course of myasthenia gravis could not be predicted precisely, since recovery or great improvement not infrequently occurred spontaneously. Thus improvement in any one patient after surgery could not be accepted as a result of thymectomy since the possibility of spontaneous improvement always existed. Naturally, if study of a relatively large number of thymectomized patients demonstrated that the course in this group differed sufficiently from the "usual course" of myasthenia gravis, a reliable decision as to the value of thymectomy could be attained. However, when we planned our study in 1941, at a time when the results only could be guessed at, this course appeared to us too hazardous, particularly when the "usual course" of myasthenia gravis was not known precisely. Of more promise in attaining a reliable result in which we and others might have confidence was the technic of using controls, whereby the course of a large number of patients with myasthenia gravis undergoing thymectomy could be compared

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with the course of a large group of such patients receiving only non-surgical treatment. We decided in favor of a controlled study, in spite of the great increase in labor and complexity entailed by the use of controls.

At about the same time studies to evaluate thymectomy were begun in a number of institutions in the United States having access to many myasthenic patients. In some centers, such as the New York Neurologic Institute[3] and the University of Michigan,[2] thymectomy was discontinued after a small series of operations had been performed because it did not appear to attain the desired result. However, further studies were done at Johns Hopkins University, the Massachusetts General Hospital and the Mayo Clinic. It is on reports of the relatively large series of cases from these institutions, particularly the ones in which controls were used, with comparison of the course in surgical cases with that in non-surgical cases, that we must lean most heavily for evaluation. For the most part, reports of single cases or of a small series of cases are of little help in view of the tendency to report favorable results and to refrain from reporting unfavorable ones. Consequently such reports have not been taken into consideration.

SUMMARY OF REPORTS EVALUATING THYMECTOMY

Keynes' Series. Keynes, a London surgeon, began early in 1942 to perform thymectomy on all myasthenic patients referred to him by physicians for that purpose unless he found some specific contraindication to surgical intervention. By 1954 he had amassed the largest series of thymectomies in the world. Under these circumstances no opportunity arose to develop a control series against which to compare the surgical results.

In a Hunterian Lecture given in 1945, Keynes[4] reported that he had operated on fifty-one patients for removal of the thymus gland and had assessed the results in thirty-three of the patients; he concluded that twenty of the thirty-three patients were greatly improved. By 1949, Keynes[5] had performed 155 thymectomies and had assessed the results in 120 patients subjected to removal of non-neoplastic thymus glands. The breakup into groups is as follows: (1) well, thirty-nine patients (32.5 per cent); (2) greatly improved, forty patients (33.3 per cent); (3) somewhat improved, thirty-one patients (25.8 per cent), and (4) no better, ten patients (8.3 per cent). Thus 65.8 per cent of these patients became well or greatly improved. Keynes reported the average age of group 1 as 28.3 years, of group 2 as 28.4 years and of group 3 as 32.1 years; he stated, "The influence of age on results is less important than at first appeared probable."

The average duration of myasthenia gravis at the time of surgery was two plus years for group 1, five plus years for group 2 and seven plus years for group 3. The average age of the patients in whom the results were classed as failures was nearly forty years, and the duration of disease tended to be long in these patients. Consequently, Keynes concluded, "It seems clear that a long history is prejudicial to recovery after operation."

Ross[6] in 1952 made an independent assessment of the results of removal of non-neoplastic thymus glands in one hundred myasthenic patients who had survived surgery by Keynes. The results were as follows: (1) quite well, forty-one; (2) greatly improved, twenty-six; (3) somewhat improved, twenty; (4) unimproved, six, and (4) dead, seven.

It is well to point out that Keynes uses the designation "quite well" as descriptive of patients who are quite well and require no neostigmine. They may be in complete remission or they may manifest minor evidences of myasthenia gravis, such as ptosis or diplopia or both. Consequently this group cannot be compared with our group in which results are designated "plus four, complete remission." If a minor degree of myasthenia gravis persists, we classify the results as "plus three, considerably improved."

By 1954,[7] when Keynes' series of surgically treated patients had reached "more than 200 patients without thymomas," his conviction regarding the value of thymectomy had not lessened and he stated in one of his conclusions that, "A large proportion of myasthenic patients are permanently and often completely relieved of their symptoms following thymectomy." Keynes has been supported by Sellors[8] of England and Dunlop[9] of Scotland who analyzed results after thirty-seven and nineteen thymectomies, respectively, and concluded that the operation was effective.

Johns Hopkins University Series. American workers have been cautious in attributing value to thymectomy and only in 1950 or thereafter did the investigators in this country publish

material indicating that they took a firm stand in favor of thymectomy.

Blalock and his associates,[1] in the aforementioned paper that precipitated the era of frequent thymectomy, said, "In concluding, we wish to emphasize again the absence of conclusive proof that the improvement noted in our patient is due to removal of the tumor from the thymic region." The second report of the Johns Hopkins University series was by the Blalock group[10] in 1941; it was concerned with six patients who underwent thymectomy, none of whom had a thymoma. Their early experience was summarized as follows: "The early results are encouraging and suggest strongly that the thymus gland is concerned in some manner in the genesis of myasthenia gravis."

In 1944, when Blalock[11] last wrote on his experience at Johns Hopkins University, he reported on twenty surgically treated patients, two of whom had thymomas. Of seventeen patients surviving surgery, one died later, three were little if at all improved, five were moderately improved, five were considerably improved and three were essentially well. Blalock's conclusions remained fundamentally conservative, although he said, "The early and sustained improvement which has been shown by some of the patients makes it difficult to escape the conclusion that thymectomy was at least partly instrumental in causing the alteration."

The next report of the Johns Hopkins University series was by Harvey,[12] in 1948. By that time thirty-two myasthenic patients had undergone thymectomy since 1941. Evidently the first case (1936) already mentioned, in which a tumor had been removed from the thymic region, had been excluded from the series, since complete thymectomy had not been attempted. Two of the patients were well and six were greatly improved. Harvey concluded that sufficient data were not yet available to allow final evaluation of the effect of thymectomy on the course of myasthenia gravis.

TABLE I
MYASTHENIA GRAVIS
JOHNS HOPKINS UNIVERSITY SERIES[13]

Sex	Treatment	Patients	Average Duration of Disease (years)	Results (%)				
				In Remission	Improved	Unchanged	Worse	Dead
Male	None	41	7.2	17	12	12	15	44
	Thymectomy	19	7.2	22	16	21	0	42
	Irradiation	19	7.2	0	42	26	10	21
Female	None	77	9.0	14	22	22	12	30
	Thymectomy	25	9.7	12	36	24	0	28
	Irradiation	21	6.8	5	38	19	19	19

The last report of the Johns Hopkins University series was made by Grob[13] in 1953, at which time he presented the results in 202 patients with myasthenia gravis, forty-four of whom had been subjected to thymectomy and forty of whom had been treated by irradiation of the thymus. The results in this series are presented in Table I. Grob's conservative conclusions are expressed in the following quotation: "The course of the patients who had thymectomy performed has been, in general, only slightly better than the course of those who had neither thymectomy nor irradiation. . . . A slightly higher proportion, particularly of the female patients, have improved, and fewer patients have become worse since thymectomy, but the difference is disappointingly small."

Massachusetts General Hospital Series. This series was first reported by Viets[14] in 1945 at which time it was composed of fifteen patients, four of whom had thymomas. Viets expressed his point of view as follows: "At the present time we are in the experimental stage."

Schwab[15] in 1949 expressed the opinion that thymectomized patients in the Massachusetts General Hospital series were progressing more favorably than would have been anticipated without thymectomy.

By 1950 Viets[16] had become convinced of the value of thymectomy in the treatment of myasthenia gravis. At that time his series numbered thirty-six patients and included seven instances of thymoma. The results in the twenty-nine patients who did not have tumors were summarized as follows: excellent, three; good to excellent, two; good, five; fair to good, two; fair, two; poor to fair, two; poor, two; dead, four, and too soon to estimate, seven. Viets did not present any data regarding specific controls and we interpreted his attitude as expressed in the main portion of his paper as being conservative in spite of his statement that "the results of thymectomy in the last nine years justify the continuation of the operation in patients with myasthenia gravis." However, an addendum to this report stated, "Since this paper was prepared, the report of Keynes (1949) on his large experience with thymectomy in myasthenia gravis has been published. Although he has approached the subject from a slightly different direction, our essential conclusions are identical. Both papers point out the value of thymectomy as a form of treatment for the disease."

Schwab and Passouant[17] in 1952 reported the results in forty-three patients who had removal of non-neoplastic thymus glands, excluding from analysis the seven thymomas in their series of fifty patients. Their results were as follows: completely cured, six; much improved, eighteen; little improved, ten; unimproved, five, and dead (operative deaths), four. Thus it was found that twenty-four of thirty-nine patients (62 per cent) who survived simple thymectomy were greatly improved, while the remission rate in fifty patients selected as controls was only 18 per cent. Unfortunately details regarding the selection of controls were not given in this report. Further analysis of their data indicated that the best results were obtained in young female patients.

TABLE II
MYASTHENIA GRAVIS
MASSACHUSETTS GENERAL HOSPITAL SERIES[18]

Results	Female				Male			
	Thymectomy		Control		Thymectomy		Control	
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
Complete remission	11	62	7	34	2	24	4	56
Considerably improved	22		11		4		10	
Improved	4	23	13	38	5	44	4	24
No essential change or worse	8		7		6		2	
Dead	8	15	15	28	8	32	5	20
Total	53	100	53	100	25	100	25	100

Schwab and Leland[18] in 1953 gave the last report of the Massachusetts General Hospital series; their data, which we consider of great importance, are summarized in Table II. Their study employed control patients who were matched with the thymectomized patients as to sex, age of onset, severity of disease and presence or absence of thymoma. The authors arrived at three important conclusions: (1) "The patients . . . who had thymomas . . . derived no evident improvement in their myasthenia from the operation." (2) "Males received no evident benefit from the operation. Thymectomy appears to be contraindicated in males in whom the onset of the disease was after 30." (3) "In females without thymomas, thymectomy appears definitely indicated."

Mayo Clinic Series. Our studies began in 1941 and the first patient of our series to have a thymectomy underwent surgery on December 20, 1941. A general survey of the series as it existed in November, 1954, is presented in Table III. Not all myasthenic patients studied at the clinic between November, 1941, and November, 1954, are included. The series has been

limited to unquestionable cases of myasthenia gravis that were studied by one of us (Eaton) except for a few surgical cases in which thymectomy was done by one of us (Clagett).

The first four papers concerning thymectomy in our series were published from August, 1943,

TABLE III
MYASTHENIA GRAVIS
COMPOSITION OF MAYO CLINIC SERIES
(472 PATIENTS FROM NOVEMBER, 1941, TO NOVEMBER, 1954)

Categories	Female	Male	Total	
			Patients	Per cent
Surgical	72	49	121	26*
Thymoma	28	23	51	42†
No thymoma	44	26	70	
Non-surgical	186	165	351	74*
Thymoma	14	12	26	7‡
No thymoma	172	153	325	
Patients (total)	258	214	472	...
Thymomas (total)	42	35	77	16*
Thymomas (per cent)	16	16	.....	...

* Per cent of 472 patients.
† Per cent of 121 surgical patients.
‡ Per cent of 351 non-surgical patients.

to May, 1949.[19–22] Recognizing the pitfalls of drawing conclusions from too few cases studied for too brief a period, we refrained in these earliest reports from making any judgment with regard to the value of thymectomy. However, by 1949 it was considered that the studies had progressed to a point justifying conclusions. At the meeting of the American Neurological Association in June, 1949, we expressed our conclusions, which were published in 1950,[23,24] that controlled studies failed to support the opinion that thymectomy was of value in the treatment of myasthenia gravis. At that time adequate follow-up data were available on seventy-two of our seventy-five surgical patients and the results were compared with 142 control patients. The results among patients undergoing surgery were somewhat better than were those for the entire group of non-surgical patients, as is shown in Table IV.

However, in this study it became apparent at once that the patients selected for surgical treatment differed significantly from the remaining non-surgical patients. Furthermore it was apparent that some of the differences might well influence the statistical studies in favor of the surgically treated patients. For example, patients who were old or were extremely ill with myasthenia gravis or complicating diseases were excluded from the group to be treated surgically and relegated to the control group. Consequently it became obvious that control patients must be selected so that they and the patients undergoing surgery would be similar with regard to age and sex, duration and severity of myasthenia gravis, and duration of follow-up.

When such selection was done, the best case that we could make for thymectomy at that time was that a truly gratifying result of surgery (complete remission or considerable improvement) was obtained in 35.5 per cent of the patients surviving thymectomy as against a 28.5 per cent chance that a comparable result would occur without surgery. (Table IV.) This difference of only 7 percentage points did not appear significant. We were sufficiently discouraged with the results of thymectomy at that time that we temporarily ceased to offer it to patients except when thymomas were present.

Later that year in September, 1949, Keynes' second report[5] appeared and endorsed thymectomy more enthusiastically than had his first report (1946). In January, 1950, appeared the aforementioned report of Viets[16] that supported Keynes in concluding that thymectomy was of great value in the treatment of myasthenia gravis. Finally, as already indicated, Ross,[6] after his independent study of Keynes' cases, joined him in 1952 in attesting to the value of thymectomy. Needless to say, we were disturbed by the differences between our results and conclusions and those of the other workers.

Consequently we and Bastron made another evaluation of our series and reported our observations at the meeting of the Association for Research in Nervous and Mental Disease in December, 1952; they were published in the proceedings of that association almost a year later.[25] In this study of 1952 every possible factor that might influence results, such as age, sex, duration and severity of disease and duration of follow-up, was taken into account so as to procure as comparable a group of control patients as possible. Furthermore we realize that in our 1949 study the group of surgically treated patients was heavily loaded with those who had thymomas. We decided to eliminate them from the study. The gist of the 1952 study is presented in Table V, in which the results in forty-four

patients surviving removal of non-neoplastic thymus glands are compared with the results in forty-eight control patients. These findings indicated that the Mayo Clinic series did provide evidence for support of the value of thymectomy. By exclusion of the patients who had thymomas thymectomized patients became worse or died, whereas 29.2 per cent of the control patients were in these categories.

In order to compare our data with those of Schwab and Leland (Table II) with regard to the influence of sex on the prognosis after thy-

TABLE IV

MYASTHENIA GRAVIS

RESULTS IN SEVENTY-TWO SURGICAL AND 142 CONTROL PATIENTS

(MAYO CLINIC SERIES, 1949)[23,24]

Results	Patients (%)			
	Unselected		Selected	
	Surgical (72)	Control (142)	Surgical (62)	Control (56)
+4 Complete remission	6.9	7.7	8.1 } 35.5	8.9 } 28.5
+3 Considerable improvement	22.2	9.9	27.4 }	19.6 }
+2 Moderate improvement	20.8	10.6	24.2	14.3
± No essential change	27.7	43.0	29.0	39.3
− Worse	2.8	6.3	3.2	12.5
−4 Died	19.4*	22.5	8.1	5.4

* Six of the deaths (representing 8.3 per cent of the surgical patients) were the result of surgical treatment.

TABLE V

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON OF RESULTS IN SURGICAL AND NON-SURGICAL PATIENTS SIMILAR AS TO AGE, SEX, SEVERITY AND DURATION OF DISEASE

(MAYO CLINIC SERIES, 1952)

Results*	Surgical		Non-surgical	
	Patients	Per cent	Patients	Per cent
+4 Complete remission	8	18.2 } 50.0	5	10.4 } 29.1
+3 Considerable improvement	14	31.8 }	9	18.7 }
+2 Moderate improvement	9	20.5	7	14.6
± No essential change	9	20.5	13	27.1
− Worse	2	4.5 } 9.0	7	14.6 } 29.2
−4 Died of myasthenia gravis	2	4.5 }	7	14.6 }
Total	44	100.0	48	100.0

* Minimal follow-up study was three and two-thirds years.

it was found that removal of the non-neoplastic thymus gland resulted in a rate of remission of 50 per cent in contrast to a 29.1 per cent rate of remission among patients in the control group. It was also found that only 9 per cent of the mectomy, we present in Table VI information regarding forty-seven of our forty-eight patients who had non-neoplastic thymus glands removed surgically and on whom follow-up data are available from our 1952 study. One patient in

whom accidental death occurred has been excluded. For comparison data are presented regarding fifty-four female and twenty-three male controls without thymomas who were comparable to the surgically treated patients in age, severity and duration of myasthenia gravis

good results in male patients. Fifty-three per cent of female patients from whom a non-neoplastic thymus gland was removed obtained complete remission or considerable improvement, whereas only 20 per cent of control female patients attained comparable status without

TABLE VI

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON BY SEXES OF RESULTS IN SURGICAL AND NON-SURGICAL PATIENTS

(MAYO CLINIC SERIES)

Results	Female				Male			
	Surgical		Non-surgical		Surgical		Non-surgical	
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
+4 Complete remission	5	53	3	20	3	35	3	30
+3 Considerable improvement	11		8		3		4	
+2 Moderate improvement	6		8		3		4	
± No essential change	4		17		5		8	
− Worse	1		14		1		2	
−4 Died (surgical death)	1	13	..	33	2	18	..	17
−4 Died of myasthenia gravis	2		4		..		2	
Total	30	..	54	..	17	..	23	..

TABLE VII

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON BY AGES OF RESULTS IN THIRTY THYMECTOMIZED FEMALE PATIENTS AND FIFTY-FOUR FEMALE CONTROL PATIENTS

(MAYO CLINIC SERIES)

Results	Patients (%)			
	Less Than 30 Years		30 to 49 Years	
	Thymectomy (22 patients)	Controls (32 patients)	Thymectomy (8 patients)	Controls (22 patients)
+4 Complete remission or +3 considerable improvement	55	25	50	14
− Worse or −4 died of myasthenia gravis	14*	38	13	27

* Includes one surgical death.

and duration of follow-up. Our data are remarkably similar to those of Schwab and Leland in that they indicate good results for female patients but fail statistically to demonstrate

surgery. Furthermore only 13 per cent of the female patients treated surgically became worse or died, whereas 33 per cent of the control female patients were in those categories.

In Table VII are presented data designed to determine whether or not older female patients, namely those from thirty to forty-nine years of age, do as well as younger ones, namely those less than thirty years of age. It would appear that the results in older women are not significantly different from those in younger female patients. Admittedly the small number of thymectomized women more than thirty years of age, namely eight in our series, prevents us from drawing definite conclusions with regard to this point but the data may be of value in reflecting trends.

It is not surprising that the sex of the patient should turn out to be a highly important determinant in prognosis after thymectomy. Pregnancy often appears to change the course of myasthenia gravis for better or worse, and it is the rule that aggravation of symptoms occurs during the days preceding menstruation. In all three of the aforementioned large American series females predominated among the younger group of patients and males among the older. In our series the patients who first noted symptoms of myasthenia gravis after the age of forty years were almost as numerous as those in whom the onset was before the age of forty. Females predominated 2:1 among the younger group and males predominated in roughly the same ratio in the older group.

Further discussion of thymectomy in the absence of thymoma will be resumed after consideration of patients in whom thymomas were present.

THYMOMA

In planning our study in 1941 for evaluation of thymectomy we decided to concentrate on patients in whom thymomas were present. This decision was influenced by the fact that the report of Blalock and associates[1] in 1939 and that of Campbell and co-workers,[26] presented at the meeting of the American Neurological Association in June, 1941, dealt with the favorable results achieved in two of three patients from whom thymomas were removed surgically. Furthermore at that time it was not illogical to presume that the removal of a large amount of thymic tissue would prove to be more effective than would removal of a small amount.

The first problem was one of roentgenologic detection of thymomas. We were unaware of any instance in which a roentgenologic diagnosis of thymic tumor had been made at the clinic prior to 1941. This was surprising in view of the fact that several hundred myasthenic patients had been examined roentgenologically. As a rule only stereoscopic films in the postero-anterior projection had been made. Abnormal shadows occasionally had been detected in the anterior mediastinum in patients who had myasthenia gravis but the roentgenologists had interpreted them as representing tumors other than those of thymic origin. This experience apparently agreed with that of other investigators. Viets and Schwab[27] in 1939 reported fifty cases of myasthenia gravis and stated, "We have been unable to associate myasthenia gravis with enlargement of the thymus gland. All roentgen ray studies were negative for enlargement of the gland." Viets in discussing the paper by Campbell and associates[26] 2 years later reported roentgenologic evidence of thymic tumor in only one of eighty-five cases.

Good[28] was challenged by the discrepancy between the frequency with which tumors of the thymus gland had been observed at necropsy and the infrequency with which they were found clinically. Consequently he undertook personal study of each of our myasthenic patients. In the first 206 consecutive patients studied a diagnosis of thymic tumor was made by roentgenologic methods in thirty-three, an incidence of 16 per cent. At last count the series had increased to 472 patients and the incidence of roentgenologic detection of thymomas had remained essentially the same. The incidence is equal in male and in female patients. The roentgenologic technic used at the clinic consists simply of use of stereoscopic films made in the postero-anterior projection followed by roentgenoscopy and usually by the exposure of films at the time of the latter examination, with the patient in the lateral or oblique projection. The accuracy of the roentgenologic diagnosis has been checked in 121 surgically treated patients and at necropsy in an additional twelve; it has been corroborated in all but two of these 133 patients. Consequently it is considered that the figure of 16 per cent is accurate to within at least a very few percentage points.

Grob[13] in the latest report of the Johns Hopkins University series stated that twelve of 202 patients had thymomas. In ten of these the course was rapidly fulminating and terminated in death regardless of whether or not thymectomy was performed. Among the forty-four surgically treated patients in Grob's series,

nine had thymomas; three of these nine died as a result of surgery and the remaining six died later of myasthenia gravis. Four patients of the non-surgical group who were found at necropsy to have thymomas had experienced rapidly progressive myasthenia gravis. Two other patients displaying roentgenologic evidence of thymoma have experienced a relatively benign course of many years' duration.

In the Massachusetts General Hospital series[18] seventeen thymomas had been encountered in seventy-eight surgically treated patients, an incidence of 22 per cent. Among the fifty-three surgically treated female patients, nine had thymomas; among the twenty-five surgically treated male patients, eight had thymomas.

Keynes[4] in 1946 reported that among his fifty-one surgically treated patients six had thymomas, one of which was malignant; two of these six patients died as a result of surgery and the others died subsequently of myasthenia gravis. Based on a study of these six patients, he stated, "Our experience suggests that when they [thymomas] occur the prognosis becomes very bad indeed."

In his 1949 report Keynes[5] eliminated patients who had thymomas from the assessment of results since "the course of the disease and the effect of operation in these patients [those who had thymomas] have been so different from the others. . . . " He went on to say, "The presence of a tumor implied a very much worse prognosis. Patients with tumors usually have a short history and severe symptoms which respond slowly and imperfectly to neostigmine."

In 1954 Keynes[7] stated that he had studied forty-one patients who had thymic tumors and that all those who had been treated surgically during the earliest part of the study had died. Of twenty-six patients who had been treated initially with roentgen rays, twenty had had the tumors removed subsequently with the following results: quite well and symptomless, four; considerably improved, eight; initial improvement, then relapse, three; no better, one, and died, four. Apparently on the basis of the results obtained in these twenty patients in contrast to the deaths occurring in all of the first fifteen patients subjected to surgery and who had not had preliminary radiation of the thymus, Keynes concluded, "The necessity for avoiding primary operation for tumors underlines the importance of carrying out a careful radiographic examination of every patient. . . . "

Data regarding fifty-five of our seventy-seven patients with thymomas on whom follow-up studies are available from our 1952 study are presented in Table VIII. For comparison Table IX presents a summary of results in all 312 patients comprising the 1952 study. The relatively poor prognosis for patients who have thymomas is reflected in these two tables. It is noted that 60 per cent of the patients who had thymomas (Table VIII) became worse or died, whereas only 39 per cent of the entire unselected group (Table IX) were in this category. If attention is paid only to the percentage of deaths, the fact that patients with thymomas fare less well is more obvious, since 58 per cent of them died as compared to deaths in 30 per cent of the entire group.

The percentage of good remissions among patients who had thymic tumors was eighteen; this is not significantly different from the 21 per cent good results for all patients comprising the study but it is in sharp contrast to the 50 per cent good results obtained in all patients without thymomas surviving thymectomy or the 53 per cent good results obtained in female patients who underwent surgical removal of non-neoplastic thymus glands. The higher death rate in the non-surgical patients with tumors is largely a reflection of the greater severity of myasthenic symptoms in this group. Most of the patients in this group were too ill to be considered for thymectomy and received roentgen therapy instead.

Although 20 per cent of the female patients who had tumors and who were treated surgically experienced satisfactory remissions, the groups were of such small size that this figure is not significantly different from the normal remission rate for female patients (20 per cent) and cannot be construed to indicate that thymectomy is of value in the treatment of thymoma. However, the fact that five of thirteen female patients with thymomas who survived thymectomy, an incidence of 39 per cent, attained desirable remissions may reflect a favorable trend. Actually, fourteen female patients survived surgery but one died of causes other than myasthenia gravis and consequently was eliminated from the study. A remission rate of 39 per cent, which is almost twice that in the female control group without tumors, cannot be interpreted as demonstrating that surgical removal of thymomas from female patients is definitely contraindicated. We believe that

more patients must be studied before definite conclusions are drawn regarding the value of removal of thymomas from female patients.

Some light is thrown on the question of malignancy of thymomas by study of this group of patients. The thymomas were considered considered to have been completely removed by resection of the invaded tissue. In three other patients the tumors were found to be adherent to but not definitely invasive of surrounding structures. Thus in this group of forty-one patients the tumors were locally invasive in fifteen

TABLE VIII

MYASTHENIA GRAVIS WITH THYMOMA

COMPARISON OF RESULTS IN FIFTY-FIVE SURGICAL AND NON-SURGICAL PATIENTS

(MAYO CLINIC SERIES, 1952)

Results	Female				Male				Total	
	Surgical		Non-surgical		Surgical		Non-surgical			
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
+4 Complete remission	3	16 } 27	0	} 10	2	10 } 15	..	...	5	} 18
+3 Considerable improvement	2	11	1	10	1	5	1	17	5	
+2 Moderate improvement	3	16	..	.....	2	10	..	...	5	..
± No essential change	1*	5	1	10	5†	25	..	...	7	..
− Worse	0		0		1	5	..	...	1	
Died (surgical death)	5	26 } 52	0	} 80	...		..	...	5	
Died of myasthenia gravis	5	26	8	80	8‡	40 } 50	5	83	26	} 60
Died of invasion of great vessels by tumor	...	.....	..	.....	1§	5	..	...	1	
Total	19	100	10	100	20	100	6	100	55	..

* Died of cerebral disease (demyelinizing); myasthenia gravis in remission at time of thymectomy; no relapse at time of death.

Note: The tumors in eight of these fourteen patients († = one of the five; ‡ = six of the eight; § = one of one) could not be removed. Patients surviving surgery received roentgen therapy.

inoperable in six of forty-one patients in whom the data have been analyzed; only biopsy was feasible because the tumor had invaded surrounding structures, including at times the pleura, lung, pericardium and great vessels at the base of the heart. The tumor in another patient was removed except for a nubbin of tissue adjacent to a major vessel. Subsequently the patient received roentgen therapy and died of myasthenia gravis; at necropsy, the tumor was found to have recurred. Another patient in whom the thymoma had been incompletely removed improved as far as the myasthenia gravis was concerned only to die as a result of recurrence of the tumor with obstruction of venous return to the heart. In seven patients the tumors, although they invaded the pleura, lung, pericardium or innominate vein, were patients and could not be completely removed in eight.

RISK OF THYMECTOMY IN MYASTHENIA GRAVIS

In deciding whether or not a patient who has myasthenia gravis is to undergo thymectomy the physician must weigh the risk to the patient of surgery against the chances of benefit from surgical intervention. In our series eight of 121 surgically treated patients died within three days of operation. Other deaths after thymectomy in this series have occurred at varying intervals but none sooner than several months postoperatively; consequently, they cannot be considered to have resulted from the operation. The over-all operative mortality rate has been 6.6 per cent.

As might be anticipated the mortality rate has been greater in patients who have thymomas, in

whom the average age is greater and the operation more difficult and prolonged. There have been five operative deaths in the fifty-one myasthenic patients undergoing surgical treatment for thymoma, a surgical mortality rate of 9.8 per cent.

The operative mortality rate in the Johns Hopkins University series was reported by Harvey[12] as three deaths in thirty-two patients; these three deaths occurred among the seven patients who had tumors. According to Schwab and Leland[11] the operative mortality rates in the

TABLE IX
MYASTHENIA GRAVIS
FOLLOW-UP RESULTS IN 312 PATIENTS
(MAYO CLINIC SERIES, 1952)

Results	Surgical				Non-surgical				Total	
	Tumor		No Tumor		Tumor		No Tumor			
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
+4 Complete remission	5	13	8	17	..	...	13	6	26	} 21 } 37
+3 Considerable improvement	3	8	14	29	2	13	21	10	40	
+2 Moderate improvement	5	13	9	19	..	...	34	16	48	
± No essential change	5	13	9	19	1	6	62	30	77	..
− Worse	1	2	2	4	..	..	23	11	26	} 39
−4 Dead*	20	51	6	12	13	81	56	27	95	
Total	39	100	48	100	16	100	209	100	312	..

* *Analysis of deaths:* (1) Eight deaths occurred as a result of surgery; five of these patients had tumors and three did not. (2) Of the patients who died later of myasthenia gravis, thirteen surgical patients had tumors and two surgical patients did not; thirteen non-surgical patients had tumors and thirty-four non-surgical patients did not. (3) Other causes of death were noted in two surgical patients who had tumors, one surgical patient who had no tumor and twelve non-surgical patients who had no tumors. (4) The cause of death was unknown in ten non-surgical patients, none of whom had tumors.

Of the seventy myasthenic patients treated surgically for removal of a non-neoplastic thymus gland, three have died as a result of surgery, a surgical mortality rate of 4.3 per cent. These three operative deaths occurred among the first thirty-two patients operated upon for removal of non-neoplastic thymus glands; since November, 1946, no deaths have been attributable to surgical treatment in the non-neoplastic group, although thirty-eight such patients have undergone thymectomy during this period. Prior to 1952 only patients who had myasthenia gravis of moderate severity were selected for surgical treatment, whereas since 1952, when we became convinced of the value of thymectomy, the condition of not a few of the patients has been such as to make the risk of operation very high but the risk was accepted on the basis that thymectomy might prove to be lifesaving.

Massachusetts General Hospital series were 7.5 per cent in fifty-three female patients and 24 per cent in twenty-five male patients. Both groups contained patients with and without tumors. Keynes[1] in 1946 reported eight operative deaths during his first fifty-one operations, two of which were among the six patients who had tumors. In 1949 Keynes[5] stated that ten operative deaths had occurred in 137 thymectomies in the absence of thymomas; five had occurred in the first eighteen operations and the remaining five in the next 119 operations. A higher mortality rate early in their experience has been the universal experience of all who have reported large series.

No useful purpose is served by comparison of the mortality rates in the various series, since details regarding the seriousness of myasthenia gravis in specific instances are lacking. Although

age, the general condition of the patient, the presence or absence of thymoma and the experience and ability of the surgical and medical teams are important factors in the evaluation of risk to the patient, experience has shown that when other factors are equal the really critical factor is almost always the severity of the myasthenia gravis. The risk of operation is directly related to the risk of respiratory failure. If the patient has experienced difficulty in breathing or in swallowing secretions in the throat, particularly if these symptoms are not eliminated by optimal amounts of neostigmine, the risk is significant. However, the performance of tracheotomy and the use of a mechanical respirator and aspirator, along with expert medical management of myasthenia gravis, usually allow the patient to survive any crisis that may be induced by operation. If the cough is so weak that the finer branches of the bronchial tree cannot be kept free from obstruction by secretions, particularly while optimal amounts of neostigmine are being administered, the risk of surgery to the patient is too great to accept and surgical intervention should be postponed.

Unfortunately medical management is difficult in severe degrees of myasthenia gravis. The chief difficulty lies, when crises arise, in deciding whether the patient has been receiving too much or too little neostigmine. In such instances we have found no substitute for tracheotomy, use of the respirator and mechanical forms of aspiration, the administration of oxygen by inhalation and close observation of the response to the administration or withholding of neostigmine.

On the other hand the risk of operation is not great provided the patient is young, can cough vigorously and has no significant difficulty in swallowing or breathing while receiving a relatively small amount of neostigmine. We would estimate the risk of death at operation in such patients as approximately 1 per cent. Of course it is well to anticipate and be prepared for trouble. Elective use of tracheotomy and the respirator is preferable to use of these aids as an emergency.

OBSERVATIONS

Use of Controls. At present, evidence overwhelmingly favors the conclusion that thymectomy is of value in the treatment of myasthenia gravis. A considerable amount of evidence is available regarding factors that have favorable or unfavorable influence on prognosis after thymectomy. Future studies promise further elucidation of the problem of selection of patients for thymectomy.

It is obvious that younger patients who have had myasthenia gravis for a short period are most likely to improve after thymectomy. On the other hand controlled studies indicate that these are the patients who are most likely to improve without thymectomy. Consequently the real value of thymectomy can be demonstrated only in control studies in which a comparison is made of the course in surgical and in non-surgical patients of the same age and in whom the duration of disease is the same. The number of patients in each group must be large enough to be statistically reliable. In this relatively early stage of our studies there is a temptation to draw conclusions from too few cases. Consequently we urge patience, persistence in study and reservation of opinion regarding the value of thymectomy in the categories that are now too small for use in the formulation of reliable opinions.

Two examples illustrate the point we are trying to make. (1) By combining the data from the Massachusetts General Hospital series and from our series, we have information regarding seventy-four female patients who had non-neoplastic thymus glands removed; these data can be compared with those from an even larger number of non-surgical controls. Complete recovery or considerable improvement occurred slightly more than twice as frequently among the surgically treated patients as among the controls. This appears to be the type of evidence deserving of confidence. (2) In our series we have follow-up data with regard to thirteen female patients who survived surgical removal of thymomas. As already stated, five patients (39 per cent) became well or greatly improved, a rate of remission which is almost twice that in female control patients who do not have thymomas. Although it is not improbable that these statistics reflect a trend favoring removal of thymomas in female patients, the question by no means can be considered settled. We believe it is well to withhold opinion under these circumstances while further data are collected and analyzed.

To summarize to this point, we can say that statistical evidence collected in controlled studies has established the value of removal of the non-neoplastic thymus gland in female patients who have myasthenia gravis. However,

the same studies do not at present indicate that thymectomy is of value as far as myasthenia gravis is concerned in males or in patients who have thymomas.

Relationship Between Myasthenia Gravis and the Thymus Gland. To clarify our attitude toward thymectomy in males, in patients who have thymomas or, for that matter, in any patient for whom the procedure has not yet been demonstrated to be of value, it is advisable to review briefly the nature of the relationship between myasthenia gravis and the thymus gland.

Weigert[29] in 1901 was the first to detect any such relationship when he described a malignant anterior mediastinal tumor at necropsy in a case of myasthenia gravis. This stimulated other pathologists to look for and describe abnormalities of the thymus gland in this disease. By 1939 the contributions of Bell,[30] Norris,[31,32] Miller,[33] and Blalock and co-workers[1] had established beyond reasonable doubt the fact that thymic tumors occurred too frequently in myasthenia gravis to be coincidental. Blalock's group were able to collect reports of fifty-three instances of abnormality of the thymus gland in approximately 110 cases studied at necropsy or surgical exploration. Of these abnormalities thirty-one were thymomas and twenty-two were instances of "persistence" or "hypertrophy."

Whatever reasons may have existed in the past for doubting the relationship between myasthenia gravis and the thymus gland have been abolished by studies of the thymus glands removed surgically during the era of frequent thymectomy. The work of Sloan,[34] Collins,[35] Bratton[36] and Castleman and Norris[37] has established that non-neoplastic thymus in myasthenia gravis is characterized not by variations in size and weight ("persistence" and "hypertrophy"), as had been claimed previously, but by histologic evidence of lymphoid hyperplasia with formation of germinal centers in the medulla. However, the frequency with which thymomas are now found in patients who have myasthenia gravis clinches the argument in favor of some sort of relationship. The study of thymomas published by Seybold and associates[38] is of signal importance and is recommended for anyone who desires to pursue the subject further.

The exact nature of the relationship between myasthenia gravis and the thymus is still under investigation. The superficially attractive hypothesis that the thymus elaborates a substance which induces the specific type of neuromuscular dysfunction recognized clinically as myasthenia gravis remains only a hypothesis. However, it has received recently its greatest support from the work of Wilson and co-workers[39] in England who found that extracts of thymus glands depress muscular contraction in various nerve-muscle preparations. The results of further controlled experiments by Wilson's group and the attempts of other investigators to corroborate their observations are eagerly awaited by clinicians. However, it appears evident that on clinical grounds alone the thymus gland does not always play a primary role in the production of myasthenia gravis since the disease at times progresses relentlessly to a fatal termination after removal of all detectable thymic tissue.

Thus clinical evidence does not favor the theory that the thymus stands in causal relationship to myasthenia gravis. A more attractive theory to us is that the basic disturbance in endocrine metabolism which probably gives rise to the specific type of muscular dysfunction recognized as myasthenia gravis also gives rise to the changes in the thymus gland characteristic of this disease. Removal of the thymus gland, when effective, may disturb the endocrine metabolic processes in such a way that they become more nearly normal with lessening of the severity of myasthenia gravis.

Thymectomy in Cases in Which Its Value Is Not Yet Demonstrated Statistically. Our reasoning in favor of continuing to do thymectomy in male patients, in patients with thymomas and occasionally even in older patients and those who have had myasthenia of long duration is based on two main concepts. First, as already indicated, a relationship exists between myasthenia gravis and the thymus gland but in all likelihood the abnormalities in the thymus do not cause myasthenia gravis. Removal of non-neoplastic thymus glands in female patients, at least younger ones, leads beyond reasonable doubt to remission of the symptoms of myasthenia gravis, probably as a result of altered metabolic processes. Second, although thymectomy has not yet been demonstrated by statistical methods to be effective in male patients who have myasthenia gravis and in patients who have thymomas, the symptoms of individual patients in these categories have gone into remission after thymectomy and there is no way of being certain that such improvement would have occurred without thymectomy. As an example, two men in our series, aged fifty-four and forty-five years,

respectively, became completely well within months after the removal of thymic tumors and had remained so for eleven and nine years, respectively, when last heard from. Another patient, a sixteen year old boy, was critically ill with myasthenia gravis and was becoming progressively worse at the time of thymectomy in July, 1954; he experienced considerable improvement within three months after surgery.

Consequently, until the time arrives when we can be certain that thymectomy will not help a specific patient or until something else of greater promise for induction of remissions is developed, we shall feel obligated to consider thymectomy for each patient. We propose to weigh the probable risk to the patient of surgical intervention against the probable chances of help, to give due consideration to all other factors that usually enter into decisions of this kind and, in each instance, to decide accordingly.

In patients who have thymomas, the locally malignant character of many of the tumors furnishes an additional indication for removal. For this reason and because of the possible beneficial effect on myasthenia gravis in female patients in particular, we intend to continue to advise thymectomy for patients who have thymic tumors, provided that either clinical or roentgenologic evidence of inoperability does not exist and the risk of surgical intervention does not appear to be excessive.

Retrospection and Prophecy. Keynes[7] has wondered why our 1949 study, published in 1950,[23,24] did not support the thesis that thymectomy was of value in the treatment of myasthenia gravis. Because our original series contained such a high incidence of patients with thymomas (33 per cent) among those who were treated surgically, that study did fail to reflect the true value of thymectomy, a fact that we were the first to recognize when doing our 1952 study. This abnormally high percentage of patients with tumors produced atypical results, and at the time there existed no good reason for suspecting the presence of such aberrant findings.

This experience makes us strongly suspect that any controlled series of cases containing a high percentage of tumors in which the results for male and female patients are not analyzed separately is unlikely to reflect the value of thymectomy when the series is relatively small. We predict that the Johns Hopkins University series eventually will support the use of thymectomy after more surgically treated patients, particularly female, without tumors are added. The better results of thymectomy compiled by Keynes may be a reflection of the highly selective composition of his surgical group. The great majority of his patients are less than forty years of age and it is in this age group that female patients with myasthenia gravis are known to predominate in the ratio of 2:1.

In 1949, as already mentioned, we did not appreciate the fact that inclusion of a high percentage of patients with thymomas could obscure the value of thymectomy. It was the reports of apparent success in the surgical treatment of three patients with thymomas that interested us in undertaking our studies in the first place. Nothing occurred during the early stages of our work to warn us that patients with tumors were to do poorly as a group. In 1947 we could report[20] that in our first fifteen surgically treated patients with thymomas, only one death, an operative one, had occurred and that seven of the eleven patients who were improved had done so to a pronounced degree. This relatively extensive experience with thymomas caused us to hesitate in the acceptance of warnings emanating from study of smaller series. The chronology of the various studies concerned also helps to explain some of the discrepancies between our earlier conclusions and those of Keynes[5] and of Viets.[16] Our 1949 study had been presented first, namely in June, 1949, at the meeting of the American Neurological Association. Keynes' study had been presented in July, 1949, as a lecture at the National Hospital for Nervous Diseases. Viets' study had been presented at the Fourth International Neurological Congress in September, 1949. However, ours was the last of these studies to appear in print.

In 1954 Keynes[7] said, "It is worth noting at this point that the Mayo Clinic had asserted a few years before that there was no convincing evidence that the thymus gland was in any way connected with myasthenia gravis." However, one of us (Eaton[40]) had proclaimed this relationship as early as 1942; the relationship between myasthenia gravis and the thymus has been discussed often by us in subsequent publications and its existence never has been denied.

SUMMARY AND CONCLUSIONS

1. The evidence found in our study combined with that in the literature makes it evident that thymectomy is of value in the treatment of female myasthenic patients who are less than

fifty years of age and who do not have thymomas. Too few patients who are alike except that they are more than fifty years of age have been studied to allow the formation of definitive conclusions. Younger female patients surviving thymectomy have better than a 50 per cent chance of attaining very satisfactory remissions, whereas without thymectomy they have a 20 to 25 per cent chance of attaining comparable results. Furthermore the chances of ultimate survival of patients in this group are substantially increased by removal of the thymus gland.

We approve of thymectomy in the case of female patients, particularly if they are young, in spite of the fact that surgery in severe degrees of myasthenia gravis is of great risk to the patient. It is probably not wise to advise thymectomy, even in female patients, if the disease has been present for many years (ten or more), particularly if it is stationary or improving.

2. The value of thymectomy in male patients has not been demonstrated conclusively up to the present time. We approve, tentatively, of offering thymectomy to male patients as of potential benefit unless the risk of surgery contraindicates the use of a procedure the worth of which is not yet conclusively established for such patients.

3. Thymectomy for removal of thymic tumors carries a relatively great risk to the patient and is not of proved value, although data in studies of the Mayo Clinic series suggest that it may prove to be of value in female patients. Perhaps it should be undertaken provided there is no clinical or roentgenologic evidence of inoperability and the risk of surgical intervention does not appear excessive. Otherwise a patient who is destined to do well as far as myasthenia gravis is concerned may die as a result of thymoma. Roentgen therapy is reserved for those patients who are unsuited for surgical treatment.

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Pregnancy in Myasthenia Gravis and Neonatal Myasthenia Gravis*

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SINCE my own experience with the problems of myasthenia gravis in pregnancy and during the neonatal period has been rather limited, I would like to introduce my presentation with some references to the literature.

Many authors have been impressed with the effect of pregnancy upon myasthenia gravis but their reports fail to serve as a reliable basis for predicting the effect in any particular instance. The occurrence of diametrically opposed reactions in relation to pregnancy adds another as yet unexplained peculiarity to the clinical course of myasthenia gravis. Perhaps most striking in this regard is the report of Laurent, who in 1931 described a patient who had had remissions during her first two pregnancies and severe exacerbations during five subsequent pregnancies, all of which terminated in abortions. In 1937 Kennedy and Moersch commented upon seven myasthenic patients who had twelve pregnancies. In five instances there was exacerbation during pregnancy with remission after termination in four; in the other seven instances there was no apparent effect during pregnancy but no mention was made of the puerperium. The most detailed study of the question of pregnancy in myasthenia gravis was reported by Viets, Schwab and Brazier in 1942. They concluded that in most cases the course of myasthenia gravis was "profoundly affected" by pregnancy, the usual course being characterized by a moderate relapse during the first trimester followed by partial or complete remission thereafter. In 1948 Harvey observed that pregnancy had a variable effect on myasthenia gravis but that exacerbations always occurred after the termination of pregnancy. In 1953 Fraser and Turner surveyed fourteen cases of myasthenia gravis in which pregnancy had occurred and concluded that there was no indication for termination of pregnancy although some tendency towards relapse was observed during the first trimester. They also reported that relapses in the early postpartum period occurred in half of their patients and apparently constituted the greatest period of danger.

It would appear that in the majority of instances pregnancy is associated with remissions during most if not all of the period of pregnancy. However in some instances, such as those reported by Wilson and Barr in 1945 and by Harris and Schneider in 1948, pregnancy may be associated with progressive exacerbation of myasthenia gravis, either with or without remission after the termination of pregnancy.

A preliminary and somewhat hasty survey of approximately 100 cases of myasthenia gravis that I have seen within the past fifteen years has disclosed four patients in whom six pregnancies have occurred. At first glance these cases would seem to illustrate the unpredictable reaction of myasthenia gravis during pregnancy. In the first case, in which the course of myasthenia gravis over an eighteen-year period had been relatively benign, the reaction during the first pregnancy was unchanged and during the second pregnancy showed only a slight exacerbation in the latter trimesters of pregnancy. In the second case the patient had had four illegitimate pregnancies and myasthenia gravis first developed three months after the termination of her third pregnancy. During her fourth pregnancy there was a rather marked remission which continued until three months after the termination of pregnancy when a slight exacerbation occurred. In the third case the course of illness during both the first and second pregnancies was characterized by initial exacerbation during the first trimester followed by a distinct remission which was maintained until two days after the termination of the first pregnancy and until eight days after the termina-

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tion of the second pregnancy. In the fourth case the course of myasthenia gravis showed a rather striking exacerbation during the last trimester with a partial remission in the postpartum period. The exacerbation in this case seemed to coincide with the probable development of hyperthyroidism. It is tempting to speculate in this case upon the possible detrimental effect of such thyroid dysfunction which has been also observed to occur in most patients in whom myasthenia gravis and hyperthyroidism coexist.

Interesting and possibly significant have been the relatively recent reports of a myasthenic syndrome affecting some infants whose mothers have myasthenia gravis. It would appear that this is an infrequent occurrence in view of the observation by Viets of only three examples in thirty-six myasthenia pregnancies. The affected infants have been considered to represent a neonatal form of myasthenia gravis in contrast to the congenital form which occurs in infants whose mothers do not have myasthenia gravis. The latter have a course of illness resembling that which is observed in children and adults; this includes an unpredictable duration, as well as exacerbation and remission. Two examples of the congenital type of myasthenia gravis were included in a series of eight children, all girls, who were reviewed in a paper which I presented at the 1954 meeting of the American Neurological Association. There have been only six other cases of congenital myasthenia gravis reported in the literature.

Twelve cases of the neonatal type of myasthenia gravis have been reported and, with the possible exception of one case, the mothers of these infants have been under treatment. TEPP was used in the treatment of one mother; the others received neostigmine. Thymectomies were performed upon three of the mothers. Myasthenia gravis was evident in these infants within a few hours to a few days after birth. The syndrome in the infants was characterized by symmetric weakness of the eyelids, face, and bulbar and skeletal musculature. The weakness was relieved by neostigmine whenever it was administered. The duration was reported to have varied from seven days to thirty-six days and in those infants who survived spontaneous and apparently permanent remission always occurred. No relapses have been reported as yet. Two of the affected infants died.

Although I have not had the opportunity of directly observing any examples of neonatal myasthenia gravis, it is probable that two of the four myasthenic mothers in my series had infants with neonatal myasthenia gravis. One of these mothers (the fourth case) had a pregnancy which terminated in the delivery of an infant who was cyanotic but started to breathe after the removal of thick mucus and application of artificial respiration. Crying was said to have been weak and death occurred one hour and thirty-five minutes after birth. Autopsy showed bronchial obstruction and cerebral congestion. There were no other abnormal findings and the thymus was apparently normal. Unfortunately this infant never received neostigmine. The other mother (the third case) has had two pregnancies and in each instance the infant apparently had difficulty in breathing and sucking. It is a noteworthy fact that the first pregnancy occurred before the mother received any treatment for myasthenia gravis. This infant's weakness, difficulty in breathing and difficulty in sucking subsided spontaneously within two or three weeks after birth. The second pregnancy terminated in May, 1954, with the delivery of an infant who had difficulty in sucking immediately after birth. This continued for six days and was relieved by the oral administration of neostigmine. In the literature the only indication of a similar occurrence of neonatal myasthenia gravis in siblings is noted in the report of Geddes and Kidd in 1951. They refer to a personal communication which described such infants following the third and fourth pregnancies of a myasthenic mother.

It has been suggested that neonatal myasthenia gravis is a result of placental transmission of a curare-like substance, the origin of which in the mother is obscure. Keynes has proposed the thymus as a source for such a substance in the mother but the reports of neonatal myasthenia gravis after thymectomy in the mother would be opposed to such a concept. If a curare-like substance is transmitted to the affected infant from the mother, it presumably is gradually excreted and destroyed and thus accounts for the apparently permanent remission if the infant survives. The question of suppression of normal neuromuscular humoral mechanisms in the affected infants because of intensive treatment in the mothers is opposed by the apparent occurrence of neonatal myasthenia gravis in infants whose mothers have not been treated.

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DISCUSSION

DR. KERMIT E. OSSERMAN: The influence of pregnancy on myasthenic symptoms and, conversely, the influence of myasthenia gravis upon gestation was studied by Drs. Nathan Kosovsky, Harold Spurt and the discusser in twenty-two myasthenic patients having a total of thirty-three pregnancies since 1940. Thirty-two per cent of the patients showed a definite remission during their pregnancy; 34 per cent showed no change in their myasthenic state, and 34 per cent showed a definite relapse associated with the pregnancy. All symptomatology patterns were usually established during the first trimester of the pregnancy and there was very little change during the second or third trimester, except in two cases. In those patients who had repeated pregnancies each pregnancy had its own influence on the myasthenia gravis and did not necessarily follow the pattern of the earlier pregnancy.

There were six therapeutic abortions and seven spontaneous miscarriages in this series. The occurrence of these spontaneous abortions bore no apparent relation to the degree of severity of the myasthenic symptoms which the patient presented. In the six cases treated by therapeutic abortions (these were the more seriously ill patients) interruption of the pregnancy did not change the severity of the myasthenic symptoms. However, following spontaneous abortion marked symptomatic improvement was observed.

Delivery and labor were normal, provided adequate cholinergic therapy was given. Cesarean section, as previously performed prior to 1937, is not required because of the myasthenia gravis but the decision to do this should be governed purely by obstetric considerations. The character of the labor was unaffected by the predelivery status of the patient. It is important to keep the patient at optimal neuromuscular function to the extent that this can be accomplished by cholinergic medication.

A three-month postpartum follow-up of most of the patients usually reflected the myasthenic status exhibited during the pregnancy. In those cases showing postpartum deterioration the added physical burden on the mother of the newborn may have been an added factor.

There are two classifications of myasthenia gravis in the newborn: (1) transient difficulty seen in children born of myasthenic mothers and (2) those rather rare cases of congenital disease in which no family history of myasthenia is obtainable. There were three occurrences of the acquired type in our series which resulted in sixteen live births. The physician should be on the watch for myasthenic symptoms in the newborn. This can be confirmed by performing either a tensilon® or neostigmine test. The baby can then be treated with cholinergic medication. The myasthenic symptoms usually abate completely in the first few weeks of life.

Endocrine Changes in Normal Pregnancy*

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PATIENTS suffering from myasthenia gravis frequently have a remission of the symptoms if pregnancy occurs, and it has been suggested that increased amounts of hormones elaborated during pregnancy may play a protective role in this disease.

Profound changes occur in the organs of reproduction during pregnancy and the body as a whole participates in many biochemical alterations in water, electrolyte, carbohydrate, fat and protein metabolism. As some of these effects are the result of increased elaboration of steroidal hormones, it seems pertinent to review some of the variations that occur in the secretion of hormones during normal pregnancy.

Gonadotrophins. In early pregnancy one of the most striking observations is the rapid rise in the concentration of chorionic gonadotrophin in blood and urine. (Fig. 1.) This substance is elaborated by the growing trophoblast for the purpose of maintaining the corpus luteum, causing it to continue to secrete progesterone and estrogens, thus preventing the collapse of the endometrial bed. Chorionic gonadotrophin appears in the urine very soon after implantation of the fertilized ovum and reaches a peak between the fiftieth and the seventieth day after the beginning of the last actually occurring menstrual period. The concentration of chorionic gonadotrophin in the blood follows a similar curve as that observed for urine.[2] The maximum level is maintained for only a short period and is followed by a rapid decrease in concentration. From approximately the one hundred tenth to one hundred twentieth day until the end of pregnancy this substance remains at a relatively low level. Following parturition it disappears from the urine within three to seven days.

Progesterone and Estrogen. The source of progesterone and estrogen in early pregnancy is the corpus luteum. This function is gradually taken over by the placenta. By the end of the second month the placenta in the human is secreting sufficient amounts of these steroids to maintain the pregnancy.

Because progesterone is rapidly metabolized in the body it is difficult to demonstrate its presence in either blood or tissue. Its chief metabolite is pregnanediol which is excreted in the urine as pregnanediol glucuronide.[3] Urinary excretion of pregnanediol in pregnancy is a reflection of the amount of progesterone elaborated. In very early pregnancy pregnanediol is excreted at the same level or slightly higher than that observed during the luteal phase of the menstrual cycle (4 to 10 mg./24 hours). Its concentration in the urine increases gradually during the first three months of pregnancy (Fig. 1), then more rapidly so that by the end of pregnancy from 40 to 130 mg. per twenty-four hours may be excreted. Following delivery of the placenta it disappears rapidly from the urine.[4]

Estrogen excretion in the urine during pregnancy follows a pattern similar to that of pregnanediol. (Fig. 1.) During the first three months it is present in the urine at levels slightly higher than those observed in the luteal phase, approximately 1 mg. per twenty-four hours. After the one hundredth day the concentration begins to increase and by the last month of pregnancy from 12 to 35 mg. per day may be excreted. The ratio of pregnanediol to estrogen in early pregnancy is roughly 10:1. In the last month it is 4:1. Thus the rate of estrogen elaboration increases more rapidly than that of progesterone. In Figure 1 is shown the interrelationship that exists between the excretion of various hormone metabolites in pregnancy.

Relaxin. Hisaw reported in 1926[5] that relaxation of the pubic symphysis in the guinea pig was under the control of a specific hormone, relaxin. Since that time it has been shown that mixtures of estradiol and progesterone are also able to induce pelvic relaxation. Relaxin can be detected in the blood of pregnant women.[6] Little is known regarding its mechanism of action.

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Adrenal Cortex. There is much evidence that points toward an intimate relationship between the adrenal cortex and the reproductive system. The increase in the size of the adrenal glands observed in pregnant women[7] represents increased activity as judged by hormonal studies of blood and urine.

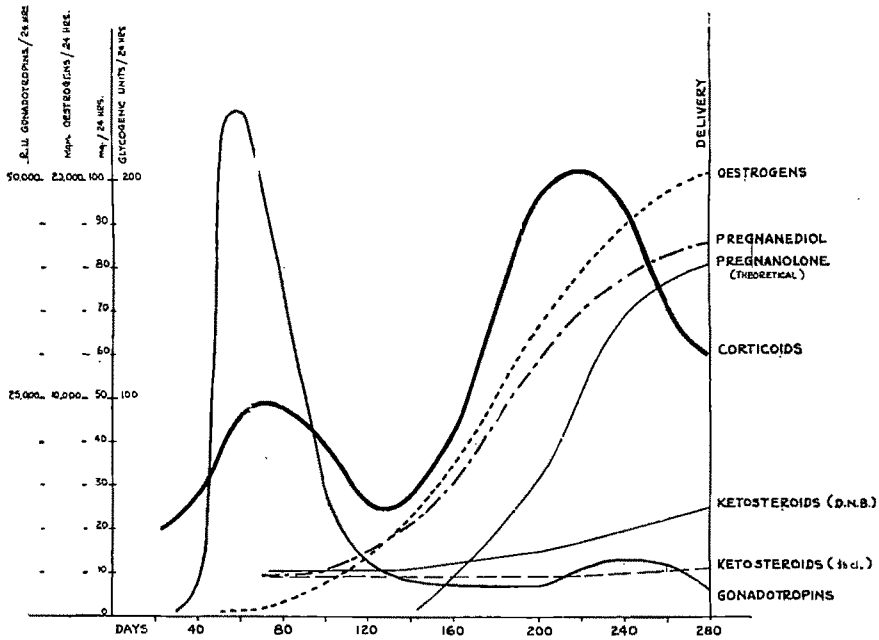


FIG. 1 Excretion of various hormones in pregnancy.

The adrenal gland elaborates a variety of steroidal hormones with different biologic activities. These include the glucocorticoids, the mineralocorticoids and a group of steroids which are metabolized and excreted as 17-ketosteroids. The glucocorticoids represent hormones such as hydrocortisone and cortisone which have a wide influence on tissue metabolism. Studies by Venning[8] show that in pregnancy there is an initial rise in excretion of glucocorticoids in the first trimester of pregnancy which usually returns to normal levels by the one hundredth to the two hundredth day. This is followed by a subsequent increase in the output of glucocorticoids which reaches a maximum excretion in the last trimester of pregnancy. This increased excretion of corticoids has been confirmed by chemical procedures.[9,10] Recent studies of the blood by Gemzell[11] showed that by the last trimester of pregnancy the blood 17-hydrocorticosteroids had increased fourfold. Venning et al.[12] reported that the average value for urinary corticoids causing sodium retention was slightly higher in the pregnant woman than in the non-pregnant woman. Little change was observed in the excretion of total 17-ketosteroids in pregnancy,[8] although it has been reported[13] that pregnant women excrete less androgen in the last month. Dobriner et al.,[14] reporting on isolation studies on the excretion of individual ketosteroids, found that the pregnant woman excretes ketosteroids which differ quantitatively and qualitatively from those found in the urine of non-pregnant women.

Thyroid Gland. In pregnancy the thyroid gland becomes more vascular and usually becomes hypertrophic, but it is not generally thought that this is accompanied by hyperfunction. An increase in the basal metabolic rate and the serum protein bound iodine[15] occurs. The significance of these observations is not clear.

Thymus Gland. Acute involution of the thymus as well as that of other lymphoid organs occurs during pregnancy. In human subjects maximal thymic atrophy is found within two months after onset of pregnancy.[16]

Selye[17] found that most hormonally active steroids will influence thymus involution in varying degrees; however, the estrogens and the adrenocortical hormones appear to be the most active in this respect. Of the adrenal corticoids the glucocorticoids have the greatest effect on thymic involution. These are the corticoids

which increase the muscle work capacity of adrenalectomized rats as shown by Ingle.[18] For some time it has been known that myasthenia gravis is frequently associated with some form of thymus lesion.

Metabolic Changes. One of the most characteristic biochemical alterations in late pregnancy is an increased retention of water. There is a progressive increase in plasma and total blood volume which becomes maximal in the ninth month. As a result of this dilution, the concentration of blood cells and haemoglobin is decreased.

It has been demonstrated that estrogens as well as adrenocortical hormones will cause water and sodium retention.[19] The period in which there is maximal water retention is associated with an increased elaboration of estrogens and adrenocorticoids. Estrogens also have a lipotrophic effect and possibly they are partly responsible for changes that occur in the total neutral fat, serum phospholipids and circulating cholesterol during pregnancy. These same hormones also affect calcium and phosphorus metabolism. Many pregnant women show a slight disturbance in carbohydrate metabolism which is due to a lowered renal threshold for glucose. Recent studies by Flynn et al.[20] on the occurrence of lactosuria and glycosuria in pregnant women showed that there is an increase in the incidence of glycosuria after the third month. It is well known that certain hormones of the adrenal cortex may affect carbohydrate metabolism and in pregnancy it is possible that the higher incidence of glycosuria may be associated with an increased adrenal function.

Various reports indicate that amelioration of symptoms in pregnant women with myasthenia gravis may occur at different periods. It is not unreasonable to suppose that the dramatic changes occurring in the endocrine glands and the consequent effects on metabolic processes in various tissues may have some influence on the course of the disease although it is not clear in what manner these remissions are brought about. In order to clarify this situation hormonal assays should be done on pregnant women with myasthenia gravis.

SUMMARY

The various changes that occur in hormone secretions during pregnancy have been reviewed. The first trimester is characterized by a rapid rise in levels of chorionic gonadotrophin in blood and urine. The function of the adrenal cortex is slightly increased at this time. In the last trimester, estrogens, progesterone (or its metabolite pregnanediol) and the adrenocortical hormones are at a high level in blood and urine. The relationship of the hormones to certain metabolic changes observed in pregnancy is discussed.

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Effect of Endocrine Glands on Normal Muscle Work*

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When the gastrocnemius muscle of a normal anesthetized rat is made to contract by intermittent faradic stimulation, it can work vigorously for several days. By feeding the rats while they are working a high level of energy output can be sustained for ten days and longer; we have kept individual animals working continuously for as long as seventeen days. The rat loses its ability to work within a few hours after removal of either the adrenal glands or the pituitary gland but it is responsive to replacement therapy with the hormones of these organs. The ability of the experimental animal to work over long periods of time can be regarded as a criterion of vigor for it is a stressful procedure which will quickly reveal physiologic deficiencies not demonstrable under resting conditions.

METHODS

The details of the procedure have been described.[1] Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they reached a weight of 200 ± 2 gm. The animals were anesthetized with phenobarbital sodium and cyclopal® sodium. The gastrocnemius muscle of the left hind leg is weighted with 100 gm. A nerve stimulator, model B (Upjohn), is used to deliver 5 pulses per second. The duration of each pulse is 20 milliseconds and the intensity is 20 ma. The distance the weight is lifted is recorded on automatic motion accumulators. Each recorder revolution represents approximately 400 gram-centimeters of work. The animals are enclosed in a cabinet with temperature constant at 28 ± 0.5°C. The test substances in aqueous solutions are injected into the jugular vein by means of a continuous infusion machine. When hormones are administered by intermittent injection into the tissues or a body cavity, the quantitative nature of the response may be affected by differences in the rates of absorption and in efficiency of utilization. The continuous intravenous injection of hormones simulates the manner in which the gland secretes its hormone into the blood more nearly than the procedures of intermittent injection. The fluid volume is 20 cc. per twenty-four hours per rat. By means of this apparatus (Fig. 1) twelve rats can be studied simultaneously.

EXPERIMENTS AND RESULTS

Studies on Adrenalectomized Rats. It has been known for a hundred years that clinical adrenal insufficiency is characterized by muscular asthenia.[2] In 1892 Albanese[3] demonstrated the muscular asthenia of adrenalectomized animals. In 1932 the author was encouraged by the studies on vigor by Dr. Roy Hoskins and his associates to test the effect of replacement therapy upon deficient work performance of the adrenalectomized rat. We observed that work performance fell below normal within a few hours following adrenalectomy and that this failure could be largely but not completely prevented by the injection of extracts of the adrenal cortex.[4] In 1934 the author joined the research group of Dr. E. C. Kendall who had supplied adrenal-cortical extracts for the initial research. When cortisone was isolated in 1935 by Mason et al.,[5] its biologic activity was immediately demonstrated in the work test, whereas it was considered to be inactive by other investigators. Subsequently 11-dehydrocorticosterone, corticosterone and hydrocortisone were isolated from adrenal cortical extracts and found to be active in this test.

When in the fall of 1938, 11-desoxycorticosterone was tested for its effect upon the work of adrenalectomized rats it was found to be

* From the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois. This work was supported by grants from The American Cancer Society as recommended by the Committee on Growth and from the United States Public Health Service.

almost completely ineffective. This compound proved to be qualitatively deficient in its biologic effect upon work and upon organic metabolism just as the 11,17-oxygenated steroids were weak in their effect upon electrolyte balance. The capacity of an adrenal-cortical steroid to support work and to affect organic metabolism is dependent upon the presence of oxygen function at position 11 on the steroid molecule. The studies of Ingle and Kuizenga[6] demonstrated that the adrenal steroids can be ranked in the following descending order of activity when assayed by the muscle work test: hydrocortisone, cortisone, corticosterone, 11-dehydrocorticosterone and 11-desoxycorticosterone.

As shown by Ingle and Nezamis[7] the continuous intravenous injection of large amounts of beef adrenal extract can sustain normal work performance in adrenalectomized rats. Doses of 15 to 20 cc. per twenty-four hours per rat were required. Full replacement has never been achieved by the intermittent injection of adrenal hormones. None of the single steroid compounds are a full substitution for either the rat's adrenal cortices or for adrenal cortical extracts. When cortisone, hydrocortisone and corticosterone are mixed together in equal proportions the peak response is not superior to that elicited by optimal doses of the single compounds. Adrenal cortical extract is superior to pure compounds in supporting work.[8] (Fig. 2.) The recently isolated steroid, aldosterone, has not been tested for this property. It seems possible, even probable, that a mixture of aldosterone and hydrocortisone would fully support work performance just as does adrenal-cortical extract.

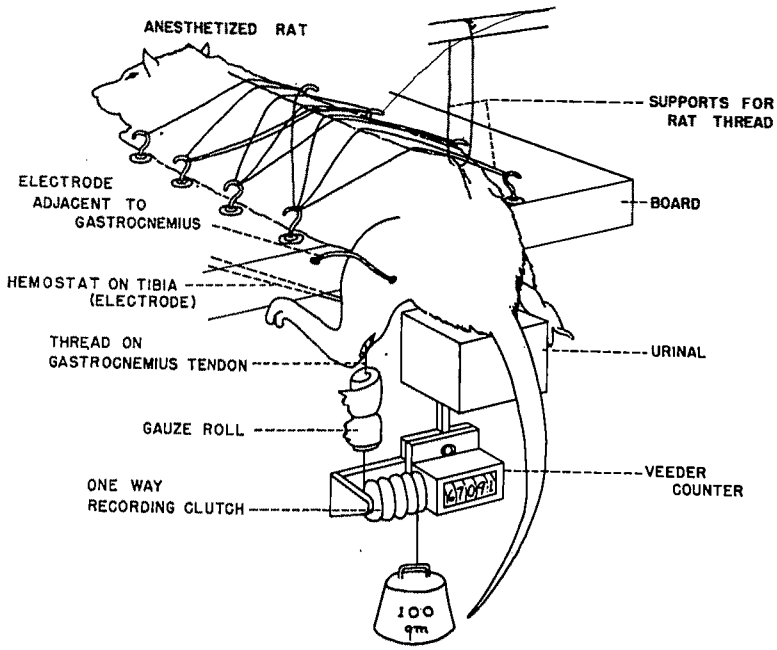


FIG. 1. Diagram of surgically anesthetized rat prepared for stimulation of muscle.

Treatment of the adrenalectomized "fa-

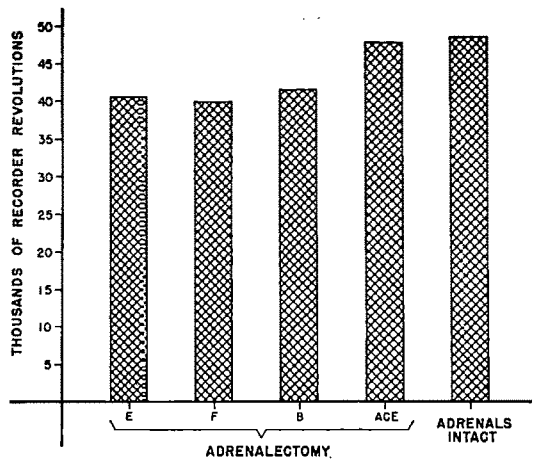


FIG. 2. Total amounts of work done by adrenalectomized-nephrectomized rats given optimal amounts of cortisone (E), hydrocortisone (F), corticosterone (B) or adrenal cortical extract (ACE) by continuous intravenous injection; average of fifteen rats per group.

tigued" rat with epinephrine will induce brief partial recovery but is incapable of prolonging the ability of the adrenalectomized rat to work. Adrenal demedullation causes the work capacity of the rat to decrease more rapidly than that of normal rats during the first ten hours, but thereafter the difference disappears and adrenal demedullated rats perform normal total amounts of work before death.[9]

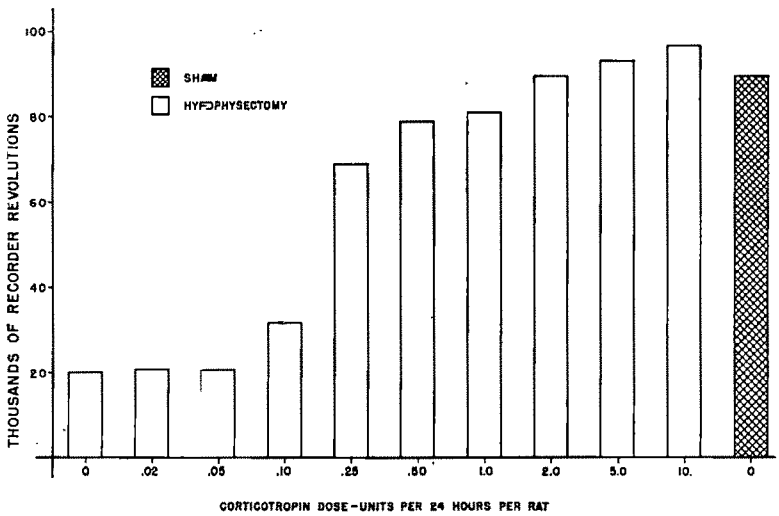


FIG. 3. Maintenance of normal work in hypophysectomized rats treated by continuous intravenous injection of corticotropin; average of fifteen or more rats per group.

Studies on Hypophysectomized Rats. The capacity of the rat to work in response to faradic stimulation is lost just as rapidly following hypophysectomy as following adrenalectomy. Indeed this failure is due in part to hypocorticalism which rapidly ensues after the source of the corticotropic hormone is removed.

Upon observing that continuous intravenous injection of commercial corticotropin sustained the ability of hypophysectomized rats to perform normal amounts of work (Fig. 3), it was supposed by us that the collapse of these animals in acute studies was due solely to hypocorticalism. To our surprise it was found[10] that adrenal-cortical extract only partially supports the work performance of either the hypophysectomized or the adrenalectomized-hypophysectomized rat, although it represented complete replacement therapy for the adrenalectomized rat. Moreover, the addition of corticotropin to adrenal-cortical extract supported normal work in the adrenalectomized-hypophysectomized rat. (Fig. 4.) At first we supposed that corticotropin itself has an important extra-adrenal effect, although we recognized that it might be contaminated with biologically important amounts of either known or unknown pituitary principles. In collaboration with Professor C. H. Li of the University of California it was shown that this "work" factor was separable from corticotropin. An optimal effect on work could be obtained with as little as 1 μg. per rat per day. Although this principle was present in all of a large number of preparations of corticotropin and other anterior lobe principles from a number of other laboratories, it could not be identified with any known anterior pituitary principle. After noting that a positive effect on work was correlated with an antidiuretic action, our attention turned to the hormones of the posterior lobe of the hypophysis. It is now established beyond reasonable doubt that vasopressin is the pituitary principle which supports the work performance of the hypophysectomized-adrenalectomized rat when added to adrenal cortical extract. This conclusion is supported by our tests of pure vasopressin* (Table 1). Further testing of the "work" fraction, which had been separated from corticotropin by Professor Li, showed that it contained approximately 2 international units of pressor activity per mg., an amount which could account for its positive effect on work. The mechanism of this effect is not known to us.

* Provided by Professor du Vigneaud of Cornell University Medical School, New York, New York.

Studies on the Diabetic Rat. We have a number of studies of the work performance of the diabetic rat, all supporting the conclusion that lack of insulin does not directly limit the ability of skeletal muscle to work or to utilize carbohydrate as a source of energy.[11]

Studies on the Effect of Castration. In studies done years ago when the conditions of the muscle work test were much less severe we failed to find any change from normal in the ability of either castrated males[12] or females[13] to do muscle work. These studies should be repeated.

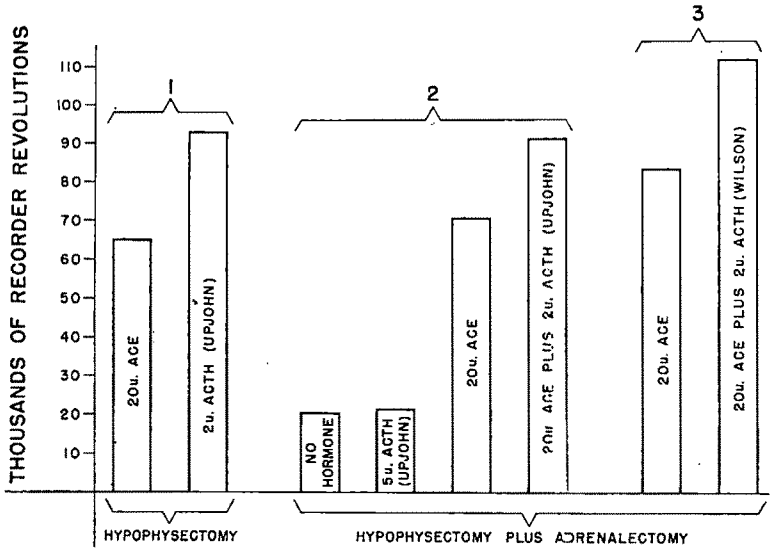


FIG. 4. The effect of some commercial preparations of corticotropin in the hypophysectomized rat and in the adrenalectomized-hypophysectomized rat with and without treatment with adrenal cortical extract; average for sixteen rats per group.

Studies on the Thyroid. Our studies on the relationship of thyroid function to vigor were of the cursory sort and have not been published. It was established to our satisfaction that the ability to sustain work output in the rat becomes subnormal during either severe hypothyroidism or hyperthyroidism. The changes from normal are much less dramatic than those caused by either adrenal cortical or hypophyseal insufficiency.

SUMMARY

When the gastrocnemius muscle of the anesthetized (phenobarbital sodium) rat is weighted with 100 gm. and stimulated to contract five times per second, it is capable of working for several days. The ability to continue work is rapidly lost following removal of the adrenal cortices or the pituitary gland. The ability of the adrenalectomized rat to work can

TABLE I

EFFECT OF VASOPRESSIN UPON WORK PERFORMANCE OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS TREATED WITH ADRENAL CORTICAL EXTRACT

(Averages and Standard Errors)

Experimental Condition	Rats	Treatment	Total Average Recorder Revolutions
Sham hypophysectomy-adrenalectomy	16	Saline only	93665 ± 3293
Adrenalectomy-hypophysectomy	25	20 cc. adrenal cortical extract first 24 hr.; 10 cc. second 24 hr.	42130 ± 3623
Adrenalectomy-hypophysectomy	16	Adrenal cortical extract as above plus 0.02 units vasopressin per 24 hr.	93885 ± 2576

be brought to within normal limits by continuous intravenous injection of large doses of adrenal cortical extract. The 11-oxygenated steroids support near normal work performance and are much more potent than the 11-desoxy steroids. However, none of the pure steroids thus far tested will support normal work performance. Aldosterone has not been studied in the work test.

The work performance of the hypophysectomized rat can be brought to within normal limits by treatment with adrenal cortical extract plus vasopressin. Vasopressin is not effective in the absence of adrenal-cortical hormones.

Insulin is not directly essential for the support of muscle work. Other hormones have not been adequately studied, although it is known that vigor declines during either severe hypothyroidism or severe hyperthyroidism in the rat.

Acknowledgment. The rats used in these studies and the adrenal cortical extract were supplied through the kindness of Dr. M. H. Kuizenga of The Upjohn Company, Kalamazoo, Michigan.

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The Effect of Certain Endocrine Glands on Myasthenia Gravis*

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SINCE Thomas Willis' first description of myasthenia gravis in 1672, each of the glands of internal secretion have been thought by some investigators to be related either to the cause or the course of this disease. The pertinent literature on this subject up to the last decade has been reviewed by McEachern[1] and Goni.[2]

It may be stated at the outset that ten years later there is still no convincing evidence that myasthenia gravis is a specific type of endocrinopathy. However, it is possible to demonstrate that certain hormonal principles of the thyroid, adrenal and pituitary glands are able to modify the altered neuromuscular transmission seen in these patients. While the precise mechanisms of this action are still obscure, it is believed that this may well prove to be a fruitful avenue of approach to an understanding of the basic pathophysiology of myasthenia gravis.

In addition to the influence of pregnancy and menstruation on this disease (which is the subject of another presentation at this conference), perhaps the most interesting relationship is between the thyroid and myasthenia gravis. In discussing this we must first eliminate those patients with "chronic thyrotoxic myopathy" and "exophthalmic ophthalmoplegia"[3,4] who do not have true myasthenia gravis. This leaves a much smaller group—perhaps two dozen or so well documented cases—in which there is coincident hyperthyroidism and true myasthenia.[5,6,34] Most of the patients have been women between the ages of twenty to fifty-nine years who have shown characteristic improvement in their myasthenia with prostigmin.® In four instances in which the myasthenia preceded the thyrotoxicosis, the onset of the latter resulted in improvement in two of the myasthenics, while treatment of the hyperthyroidism made the myasthenia worse. This interesting "see-saw" relationship, originally commented on by McEachern,[5] has also been recently described by Maclean and Wilson.[6] Levitt[15] urges combined thyroidectomy and thymectomy in these cases and reports 'good results" in six patients with thyrotoxicosis and myasthenia gravis so treated.

The cases described by Laurent[7] and Waldenström[8] and designated as "acute thyrotoxic bulbar palsy" or "acute thyrotoxic encephalomyopathy" are quite likely fulminant cases of myasthenia gravis associated with hyperthyroidism. That there is some "overlap" between these and the previously described cases is indicated by the sudden death (in respiratory failure) of three of twenty-one reported cases of "chronic" thyrotoxic myopathy[3,9,10] and the observation of McEachern and Ross[3] that prostigmin produced significant improvement in some but not all of these same cases. Finally, the interesting association between hypothyroidism and myotonia, the "mirror-image" of myasthenia gravis as far as neuromuscular transmission is concerned, may be mentioned.[11]

Three interesting laboratory observations are worth mentioning in connection with the thyroid gland Tickner[12] is said to have found significant increase in serum cholinesterase levels in 50 per cent of thirty-five patients with thyrotoxicosis. Rawson[13] has reported that the thyrotropic factor (TSH) of the adenohypophysis is inactivated not only by iodides and thyroxine but also by thymus tissue. Finally, focal collections of lymphocytes ("lymphorrhages") are quite common in extraocular and somatic muscles in hyperthyroidism as well as in myasthenics.[14]

Nevertheless it is the rare patient with myasthenia gravis who will show any obvious clinical signs of thyrotoxicosis or any demonstrable alteration in the basal metabolism rate or serum cholesterol values. If there is a report of

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investigation of radioactive iodine uptake or serum protein-bound iodine studies in a series of myasthenia gravis patients, we are not familiar with it.

Coincidence of adrenal cortical insufficiency and myasthenia gravis is even rarer. In one such case personally reported[16] it was of interest that the patient failed to respond convincingly to prostigmin until fluid, carbohydrate and electrolyte values approached normal. Clinicians dealing with myasthenics are familiar with their poor tolerance to stress of almost any kind. Non-specific changes in the adrenal cortex have been described in myasthenics and a few enthusiastic reports of the efficacy of various adrenal extracts are on record. Sloan[17] found that the thymus gland in three of seven patients with Addison's disease showed changes similar to those seen in myasthenia gravis. Finally, Thevenard and Leger[18] have recently described improvement in four myasthenic patients subjected to denervation of both carotid sinuses, a procedure which in dogs is said to result in adrenal cortical hyperplasia.

Just how to integrate the diverse observations previously cited is a problem, but it is of considerable interest that thymic hyperplasia occurs in both Addison's disease and Graves's disease (as well as in castrates). Whether or not the thymus may provide the missing link, the overall situation currently is still very obscure and in critical need of reinvestigation by the powerful newer technics which the endocrinologist has developed in the past decade.

TABLE 1

SUMMARY OF REPORTED RESULTS OF TREATMENT OF MYASTHENIA GRAVIS WITH CORTICOTROPIN (ACTH) AND CORTISONE

Authors	Total Treated	No. Improved	Marked Improvement	Slight to Moderate Improvement	No Change or Worse
ACTH					
Schlezinger[22]	10	10	10	0	0
Torda and Wolff[23]	15	10	10	4	1; died third day
Wolfson[24]	4	3	2	1	1
Shapiro[25]	3	1	1	0	2; 1 died thirteenth day
Millikan and Eaton[26]	2	1	0	1	1
Soffer[27,28]	2	1	0	1	1
Stanbury[29]	1	1	1	..	.....
Sprague[30]	1	1	..	1	.....
Glaser[31]	1	1	1	..	.....
Grob and Harvey[32]	10	0	0	0	10
Ritter and Epstein[33]	1	0	0	0	1
Cortisone					
Millikan and Eaton[26]	3	1	0	1	2
Grob and Harvey[32]	3	0	0	0	3
Schlezinger[22]	1	1	0	1	0

The third chapter in the "endocrine story" of myasthenia gravis became possible in 1943 with the isolation of the adrenocorticotropic factor (ACTH) of the anterior lobe of the pituitary in "pure" form in appreciable quantities by two groups of workers.[19,20]

Corticotropin was first used in the treatment of myasthenia gravis by Torda and Wolff[21] at the New York Hospital in 1944. They gave the following rationale for its use in these cases: (1) removal of the pituitary gland in rats induces changes in the electromyogram which closely resemble those seen in patients with myasthenia gravis; (2) the pituitary glands of several patients dying of this disease have shown an eosinophilic colloid material "suggesting altered function"; (3) administration of corticotropin (ACTH) causes shrinkage of the thymus and enhances synthesis of acetylcholine.

Corticotropin first became available commercially in 1948. Since then, a dozen references have been found concerning the use of it

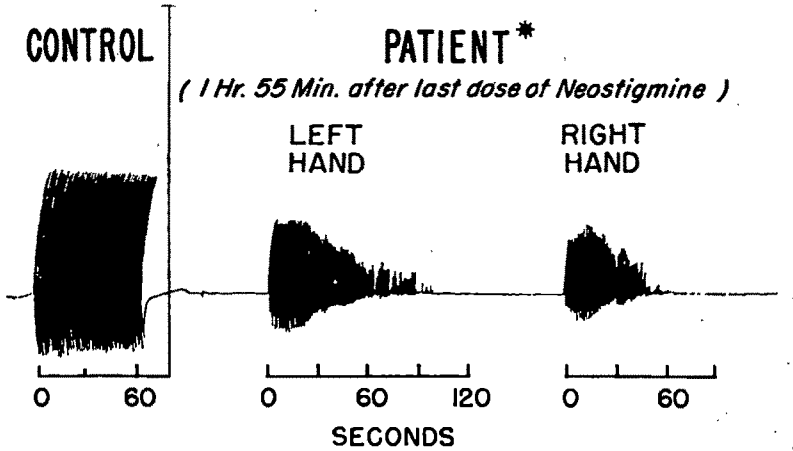


FIG. 1. Myogram: first day of ACTH therapy (unpurified) (40 mg. intramuscularly every six hours for eight days). Technic: all records taken at same interval following neostigmine. Recordings on Bell kymograph; speed 2.3 cm./min. Patient asked to squeeze bulb maximally once a second.

*Patient confined to bed, receiving 3 mg. neostigmine (H) every two hours.

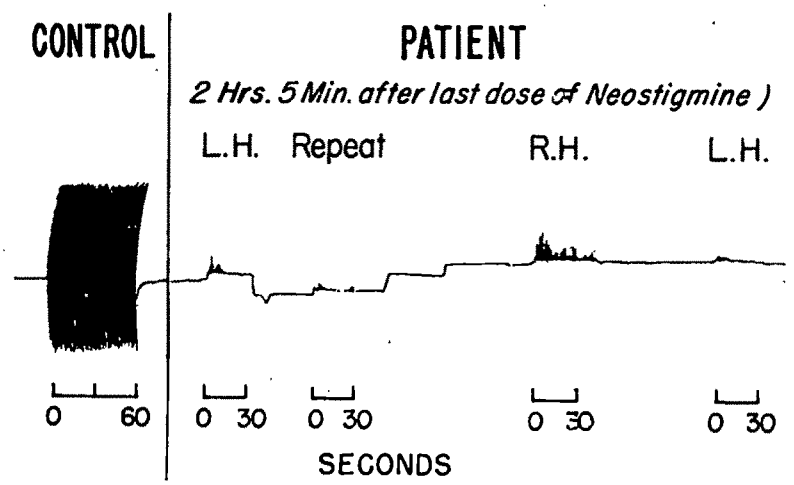


FIG. 2. Three days after ACTH.

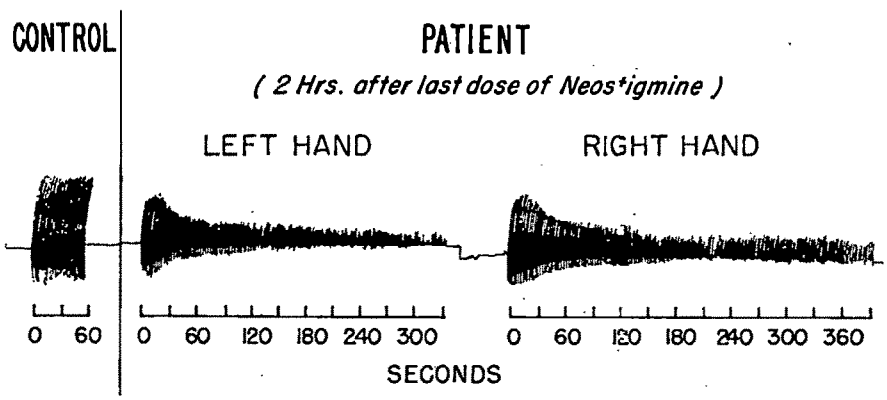


FIG. 3. Thirty-four days after ACTH.

(and cortisone) in sixty patients with myasthenia gravis. The reports are summarized in Table 1 and a typical response to corticotropin in a severe myasthenic is shown in Figures 1 to 4.

CONCLUSIONS

On the basis of a study of these reports and our personal experiences with the use of various

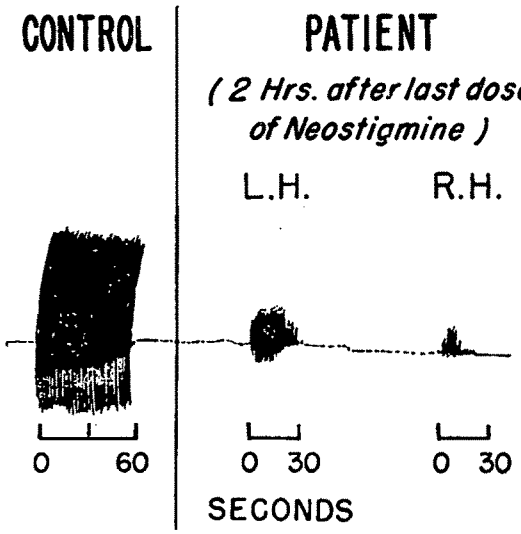


FIG. 4. Fifty-four days after ACTH.

pituitary fractions over the past three years, the following general conclusions seem justified: (1) Neither cortisone nor corticotropin is a panacea for myasthenia gravis; (2) the results with cortisone are disappointing and probably warrant little further study; (3) the results with corticotropin indicate, as a general pattern, initial worsening, improvement varying from slight to striking, beginning usually at the termination of therapy and lasting usually a matter of weeks and (4) despite the two deaths occurring during corticotropin administration to gravely ill patients, the results (over 50 per cent "markedly improved") cannot be disregarded in the light of our currently limited therapy.

The basic question, then, to be answered by future, better controlled investigations is not "does corticotropin help some myasthenics," but "how does it work?" and "could the pituitary-thyroid-adrenal axes be primarily responsible or contributory and not the thymus?" A coordinated "team-approach" combining the disciplines of the electrophysiologist, neurologist and endocrinologist will have to be achieved if further progress is to be made in this field.

Meanwhile, it is our personal belief that corticotropin still has a definite if distinctly limited contribution to make in treating myasthenic patients. We would define this as follows: (1) not justified in the mildly or critically ill patient; (2) best used in moderately severe myasthenic patients especially those with a radiologically enlarged thymus. In these patients the clinical and x-ray response may furnish a useful prognostic guide in the event of anticipated surgery (thymectomy); (3) treatment must be in a hospital equipped with adequate facilities for special tests and the care of respiratory emergencies.

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WIN 8077 in the Treatment of Sixty Myasthenia Gravis Patients*

A Twelve-month Report

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SINCE the successful introduction of oral tablets of neostigmine bromide in the treatment of patients with myasthenia gravis there has been a continued search for a better substance. One disadvantage of neostigmine is its short action; patients with severe

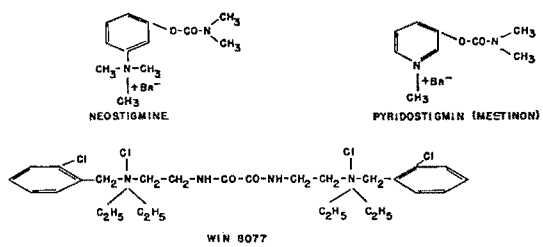


FIG. 1. Structural formulas of neostigmine, mestinon and WIN 8077. (From *J. A. M. A.*, 158: 625, 1955.)

cases have to take the medication every hour or so throughout a twenty-four-hour period. Even when this is achieved there may be a tendency for the drug to lose its effectiveness toward the end of this short interval. Furthermore, some patients are sensitive to this drug so that they have muscarinic effects of the cholinergic substance when the dose is increased sufficiently high to neutralize the symptoms of myasthenia gravis.

The use of belladonna preparations with each neostigmine dose, which would neutralize these unpleasant side effects, carries with it a certain risk. Dangerous toxic side effects can result when the warning signs of intestinal stimulation are thus hidden.

In November, 1953, an entirely new preparation[1,2] was made available for experimental trial by the Sterling-Winthrop Foundation. This preparation, called WIN 8077,† is N,N'bis(2-diethylaminoethyl) oxamide bis-2-chlorobenzyl-chloride, whose structural formula can be compared with neostigmine and pyridostigmin. (Fig. 1.) This compound is a white crystalline powder with a molecular weight of 608 and a melting point of 184.3°C. It is very soluble in water and can be sterilized by heat without alteration of its properties. It has a strong anticurare effect which is ten times that of tensilon and two to three times more than that of neostigmine and is of longer duration. Its anticholinesterase activity is nearly the same as neostigmine. No chronic toxic effects to the kidneys, liver or blood-forming organs were found in animal toxicity studies. Overdosage produced the same effects in animals as neostigmine, namely, vomiting, diarrhea and salivation. A preliminary report of its trial in myasthenia has already been made.[3]

TECHNIC OF EVALUATION

Like any new compound which has no known dosage levels in patients, the preliminary testing involved the use of minute amounts of the substance. Intravenously, in doses under 50 μg., no demonstrable effect on a patient with known myasthenia gravis was noted. It was not until the intravenous level of 250 μg. was reached that definite antimyasthenic effect was obtained. With this information oral trial of the medication was started in a number of patients in order to determine an effective dosage level. The first

† This drug is now being processed for release by the Food and Drug Administration (January, 1956) under the name of mysuran®.

* From the Neurology Service and Myasthenia Gravis Clinic of the Massachusetts General Hospital, Boston, Mass. This investigation was supported by a grant-in-aid from Winthrop-Stearns, Inc., New York, New York, who kindly supplied the material used in this investigation.

form of the preparation WIN 8077 was a syrup. This allowed a gradual increment of dosage without the necessity of working with fractions of tablets. We found that some patients had a definite response with 5 mg. per dose while others required as much as 50 mg. With this information available we recommended the clinical evaluation of 10 mg. scored tablets and a syrup containing 3 mg./cc. Patients who were adequately adjusted to neostigmine were transferred to the new preparation tentatively substituting 7½ mg. of WIN 8077 for 15 mg. of neostigmine bromide. The substitution was gradual at first so that patients continued to take both drugs during the period of adjustment. We found that this technic was not desirable since the two drugs seemed to have different lengths of action and there was a tendency for toxic side effects to occur too readily with this method. Subsequent changeovers involved abrupt switching of the patient to the new compound and cessation of the administration of neostigmine. This method proved more satisfactory and did not involve the patients in undermedication for more than a day or two.

Criteria for Effectiveness of the New Drug. A five-point numerical evaluation with 4, 3, 2, 1, 0 was used for scoring the value of this new compound. A top score of 4 goes to a drug which is objectively effective against symptoms in a clear and certain manner when taken by a patient in the absence of any other drugs. However, if the drug produces side effects requiring the presence of another drug to neutralize them the clinical value would be only 3. In this category it is assumed that the beneficial effect of the second drug is only associated with neutralization of the side effects of the first, and has no specific properties against the symptoms of the disease. A score of 3 also requires unequivocable and objective evidence of effectiveness. Category 2 applies to drugs which objectively assist another drug to improve the patient. Category 1 contains drugs that have a doubtful effect when linked with active compounds or a largely subjective one reported by the patient. Category 0 contains drugs with absence of both subjective and objective data as evidence of effectiveness.

In the study of WIN 8077 a sufficient number of patients taking only this preparation with definite objective improvement place this substance in Category 4.

Side Effects. Side effects from WIN 8077 are generally no different from those of neostigmine and other cholinergic drugs used in the treatment of myasthenia gravis. Patients who are sensitive or receive too much of the drug complain of excessive salivation, cramps, intestinal peristalsis, diarrhea and frequency of urination. These muscarinic effects are less likely to occur than with neostigmine bromide. The excessive salivation with WIN 8077 occurs before cramps and diarrhea, suggesting that the drug is more specifically powerful as a secretory stimulant. Dangerous side effects associated with the beginning of cholinergic block occur with this drug sometimes before cramps and diarrhea are evident. These are slowing of the pulse, myosis of the pupils, fasciculatory twitchings of the muscles of the tongue, shoulder or arms, and gradual onset of weakness of all muscles.

Precautions in Administration. It has been found that this drug has roughly twice the effectiveness per milligram of neostigmine in reducing the symptoms of myasthenia gravis, and approximately twice the duration. This prolonged effect may persist for as long as six hours. It is for this reason that the use of this preparation requires more care than neostigmine in the avoidance of side effects. It is better to undermedicate the patients for a number of days in switching them over from neostigmine than to risk overstimulation which may be dangerous. When given parenterally, either intravenously or intramuscularly, WIN 8077 exerts the same prolonged effect as by mouth. Therefore, particular caution must be used with an injection of this preparation since the effect may last several hours, making such overdosage doubly hazardous. The author recommends that the drug not be used parenterally, without special precautions, until more data can be obtained. For further evidence of overdosage the cautious use of intravenous tensilon,® 2 mg., may be tried. A transient increase in symptoms confirms cholinergic overdosage.

In any case of overdosage with WIN 8077 the prompt use of intravenous atropine sulfate, 1 mg., temporary cessation of all cholinergic therapy, and use of a respirator if necessary may be life-saving measures.

CASE REPORTS

CASE 1. A twenty-seven year old man with myasthenia gravis of two years' duration, involving ptosis and dysphagia, was successfully adjusted on neostigmine bromide (eight tablets per day). After a number of weeks an itching,

papular rash developed which bothered him considerably. This rash disappeared when the neostigmine bromide was removed. The patient had previously been sensitive to bromides by mouth. The physician in charge was at a loss to know any way in which the extreme sensitivity to bromine could be overcome in order that the patient's myasthenia gravis could be brought under control. WIN 8077 was substituted, five 10 mg. tablets per day, and excellent neutralization of his myasthenia resulted. No skin reaction occurred and the patient has been receiving WIN 8077 for the past six months. This is an unusual sensitivity to the bromine in neostigmine bromide but it does illustrate one of the advantages of WIN 8077.

CASE II. A forty-three year old woman with myasthenia gravis for five years, involving ptosis and diplopia as well as general weakness, was extremely sensitive to adequate doses of neostigmine bromide. She had diarrhea and cramps if the dosage was at a therapeutically effective level. Mestinon bromide was substituted for neostigmine bromide and the gastrointestinal effects were not present, but the antimyasthenia effect of this preparation in this particular patient was not as satisfactory as with neostigmine. She still complained of considerable weakness and diplopia when taking twelve 60 mg. tablets of mestinon bromide per day. She was changed over to WIN 8077 and immediately reported the same benefit she had had with neostigmine but without disagreeable gastrointestinal side effects. She was regulated on eight 10 mg. tablets of WIN 8077 per day without the appearance of any diplopia during the twenty-four-hour period. She has been maintained on this preparation for twelve months without any need of altering the dosage or increasing the amount per day.

CASE III. A forty-seven year old woman in two months developed bilateral ptosis, diplopia and some general weakness. An ophthalmologist diagnosed myasthenia by the prostigmin test which produced quite a severe gastrointestinal upset. A tensilon test confirmed the diagnosis without any side reactions. The patient, because of her reaction to neostigmine, was started on WIN 8077 in liquid form, 3 cc. of the elixir four times a day, with excellent control of her ocular symptoms. When she tried the 10 mg. oral tablets of WIN 8077 she had cramps, nausea and anorexia. With the elixir for a second time, 9 mg. per dose (3 cc.), there were no side effects. These recurred when tablets were tried once more. She is now comfortably regulated with the liquid. It is difficult to be sure whether the 1 mg. difference in the dose or the physical form of the preparation is responsible for this marked difference in tolerance. Some patients do much better on liquid medication and this case emphasizes the need of such a form for its administration.

CONCLUSIONS

WIN 8077 is a new effective substance to use in the treatment of myasthenia gravis. It has been employed successfully in forty of a total of sixty patients during the past 12 months. The side effects are similar to neostigmine and mestinon but are less likely to produce gastrointestinal disturbances when the therapeutic dosage is reached. It has a longer action than neostigmine and therefore it must be used with caution. Its parenteral use is not advised at this time.

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Progress Report on Mestinon Bromide (Pyridostigmine Bromide)*

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NEOSTIGMINE has withstood the test of time and has long been the undisputed drug of choice for the treatment of myasthenia gravis.[1] It has been effective in the treatment of the great majority of patients with this disease, especially those with the milder types of myasthenia gravis for whom relatively small doses of neostigmine are necessary.

The two chief disadvantages of neostigmine are its undesirable side effects such as gastrointestinal distress and its relatively brief duration of action. A third and most important disadvantage is that resistance to neostigmine may occur in severe myasthenia, so that even with increased dosages one cannot completely reverse the marked weakness of the patient.

There are many new cholinergic drugs available for research in myasthenia gravis today. One of these is mestinon® bromide (pyridostigmine bromide), † which is a dimethyl carbamate of 3-hydroxy-1-methyl pyridinium bromide. Preliminary results obtained with mestinon in the Mount Sinai Hospital Myasthenia Gravis Clinic were recently reported.[2] At the present time there are 283 patients reported in the American literature[3] and fifty-six in the European literature[4] who have received this drug. There are approximately 750 to 1,000 patients throughout this country who are now receiving mestinon therapy.[5]

In evaluating any new drug therapy for myasthenia gravis, we believe the following criteria must be considered: (1) What is the duration of action of the drug? (2) What relief of myasthenic symptoms does it offer? (3) Is the drug non-toxic? (4) Can the drug be helpful when the patient is resistant to neostigmine?

† The mestinon bromide used in this study was supplied by Dr. Thomas C. Fleming of Hoffmann-La Roche, Inc., Nutley, N. J.

METHODS

In the past year and a half eighty-one patients from the Myasthenia Gravis Clinic of the Mount Sinai Hospital have been treated with mestinon. The age and sex groups of these patients are listed in Table 1. The youngest age was thirty-eight weeks and the oldest eighty-three years.

TABLE 1
AGE AND SEX DISTRIBUTION OF EIGHTY-ONE PATIENTS

Age (yr.)	Male (25)	Female (56)
0–10	..	4
11–20	2	3
21–30	..	10
31–40	4	24
41–50	6	9
51–60	8	4
61–70	2	2
71–80	2	..
81–90	1	..

The severity of myasthenia gravis in a patient was estimated on the basis of cholinergic medication needed, but was classified also according to the economic usefulness of the patient to the community. Severe cases were considered to be those patients so affected by their myasthenia gravis that, despite therapy, they were bedridden or so weakened that they were unable to work; moderate cases were those patients who took relatively large doses of cholinergic medication in order to function and required frequent periods of rest during the day; and mild cases were those patients who maintained their economic place in society, were fully employable, attended school, or performed housework regardless of dosage of cholinergic medication, usually comparatively small. Our group of eighty-one patients before treatment with

* From the Myasthenia Gravis Clinic and the Department of Medicine, The Mount Sinai Hospital, New York, N. Y.

mestinon fell into the following groups: severe, twenty-four; moderate, twenty-one; mild, thirty-six.

Patients were admitted to the hospital for transfer from neostigmine to mestinon and the technic of serial tensilon® testing[5] was used to secure a quantitative comparison between neostigmine and mestinon in the same subject and to determine any difference in the duration of action of the two drugs. Subsequently, patients were given mestinon in the Clinic, merely substituting a 60 mg. tablet for each 15 mg. neostigmine tablet. More recently we have started new patients directly on mestinon therapy in the Clinic.

All of our patients have been studied by routine x-rays of the chest for thymic enlargement, electrocardiography, radioactive iodine and blood iodine studies, frequent blood counts including platelet counts and, in many cases, bone marrow examinations. In addition, routine urinalysis and physical examinations are performed at each clinic session. The effect of mestinon on the hematopoietic system was thoroughly investigated. We have found no evidence of any alteration to date.

The results of serial tensilon testing showed that the duration of action of mestinon is only slightly more protracted than that of neostigmine (approximately one-half hour longer). Mestinon cannot be considered a longer-acting cholinergic medication. The frequency of its administration during the day shows no appreciable advantage over neostigmine.

However, many patients, particularly those in the group with severe symptoms, were most gratified in that they could arise in the morning without severe myasthenic symptoms. This was most important to those patients receiving neostigmine who could not swallow their first morning dose because of dysphagia and therefore had to resort to an injection of neostigmine methyl sulfate to start the day. This nocturnal relief with mestinon has become increasingly apparent with further transference of patients so that now we do not prescribe mestinon during sleeping hours except on extremely rare occasions.

Mestinon is more effective than neostigmine in the relief of the small muscles innervated by the cranial nerves. Particular relief was noted in the muscles involved in ptosis, diplopia and dysarthria.

A striking advantage of mestinon over neostigmine is in the marked reduction in the incidence of muscarinic side effects such as epigastric distress, abdominal cramps and diarrhea, and in the relief of nicotinic side reactions such as skeletal muscle cramps. This reduction in side reactions was so pronounced that atropine could be discontinued in 75 per cent of the sixty-eight patients receiving atropine with neostigmine.

TABLE II

RESULTS BASED ON PRODUCTIVE CAPACITY IN EIGHTY-ONE PATIENTS

Drug Administered	Severe	Moderate	Mild
Original medication:			
Neostigmine	24	20	33
None	0	1	3
Final medication:			
Mestinon	8*	12	53
Neo-mestinon combination	0	1	2
WIN-mestinon combination	2	2	0
WIN 8077	...	..	1

* Including five deaths.

Schwab has commented on the possibility that in the absence of the early warning symptoms of overdosage special attention would be necessary to avoid cholinergic effects. We found no difficulty on this account because the usual muscarinic side reactions occurred as soon as overdosage of mestinon was reached. In fact, it is easier to avoid overdosage with mestinon than with neostigmine because of the wider range between therapeutic and toxic effect. Of course, in any question of doubt resorting to a tensilon test quickly resolves the problem.[7] (Table II.)

Upon transfer to mestinon the twenty-four patients who were considered severely incapacitated while treated with neostigmine showed such an improvement that fourteen of them could be reclassified in the moderate or mild group. Of twenty-one patients who were originally in the moderate group, six were so improved as to fall into the mild group. Thus, 45 per cent were improved enough to overcome their economic liability to their families and to the community.

Thirty-one patients had excellent results in that their myasthenia was improved. Twenty patients had good results in that improvement in myasthenic symptoms occurred but was not as notable as in the excellent group. Eight patients found no difference in the action of neostigmine and mestinon, and five patients preferred neostigmine to mestinon. In addition, there were four remissions in the group of eighty-one patients. (Table III.)

Some patients in the severe group, when first

transferred to mestinon, did not seem to be improved in any noticeable degree. However, with the continued use of mestinon over many months, there was a gradual but marked improvement in these patients. Of course, in a disease characterized by remissions one is reluctant to attribute the improvement to a drug. Nevertheless, the type of improvement seen was not that typically observed in a remission.

TABLE III
COMPARATIVE RESULTS OF MESTINON TO NEOSTIGMINE BASED ON IMPROVEMENT IN MYASTHENIC SYMPTOMS

Results	Mestinon	Neo-Mestinon Combination	WIN 8077-Mestinon Combination	WIN 8077
Excellent.....	31	2	1	1
Good.....	20	1	2	..
No difference..	8	..	..	..
Poor.....	5	..	1	..
Deaths.....	6*	..	..	..
Remissions....	4	..	..	..

* See text regarding one death which appears twice in table.

Attempts were made to improve the effectiveness of mestinon in patients in whom an excellent result was not obtained. Ephedrine sulfate, 3/8 to 3/4 gr. (25 to 50 mg.), was added to the maintenance dose of mestinon without any apparent improvement.

A number of patients were given a mixture of neostigmine and mestinon on a one-to-one or one-to-two basis in an attempt to increase limb strength and to give the "lift" which they desired. We were able to achieve two excellent results and one good one using this combination.

In view of a marked increase in limb strength noted with WIN 8077,* we tried this drug alone and in combination with mestinon in twelve patients. One excellent result was obtained with WIN 8077 alone and one excellent, two good and one poor result with combined WIN 8077 and mestinon therapy. We have no evidence of any synergistic action of neostigmine and mestinon, and apparently none between WIN 8077 and mestinon.

To the present time we have not found mestinon, WIN 8077 or any mixture of drugs effective when a patient is resistant to neostigmine.

There were six deaths in myasthenic crisis in the severe group; one of these occurred when a patient was admitted to another hospital and was not recognized as a myasthenic. Cholinergic medication therefore was not given.

Our mortality rate is 6.3 per cent. There have been instances when acute myasthenic crisis was relieved by mestinon, due to the marked decrease in side reactions which permitted the use of larger doses of mestinon without severe and uncomfortable muscarinic distress.

COMMENTS

We have tried to answer the four criteria proposed in evaluating any new drug therapy for myasthenia gravis: (1) As to the *duration of action* of mestinon, we find it only slightly longer-acting during the diurnal hours and therefore of no appreciable advantage over neostigmine. However, the effect during the nocturnal hours is much more prolonged with mestinon, permitting the patient a full night's sleep. (2) Mestinon gives marked *relief from myasthenic symptoms*, particularly those of the bulbar type. Results were excellent or good in 72 per cent of our series. Forty-five per cent of our patients in the severe and moderate groups were so improved as to overcome their economic liability. (3) Mestinon is definitely *less toxic* than neostigmine; this is one of its most striking advantages. (4) Through its lack of toxicity mestinon can be *helpful on occasions when the patient is resistant to neostigmine.* However, as yet we have no drug or combination of drugs which is effective when a patient in a myasthenic crisis is resistant to neostigmine.

Although mestinon is not the long-acting drug that we hoped to find, it is a valuable step forward in the search for the ideal drug in the treatment of myasthenia gravis and for the reasons already given, a superior drug to neostigmine. We still need a drug that is long-acting for the entire twenty-four hours of the day and one in which resistance does not develop. This search will be continued.

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* Mysuran.®

Management of Myasthenic and Cholinergic Crises

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THE term crisis, as applied to myasthenia gravis, usually implies failure of muscles concerned with respiration and/or deglutition to such an extent that neither function can be maintained without mechanical aids. A true myasthenic crisis may occur in an untreated or insufficiently treated patient with severe myasthenia. Apparent resistance to antimyasthenic drugs develops in an occasional patient, resulting in crisis.

Only recently have we recognized that cholinergic drugs in excess can bring about a crisis.[1] This cholinergic crisis may be difficult to distinguish from true myasthenic crisis. It is possible that many patients previously thought to be in crisis due to drug resistance were actually in cholinergic crisis.

Grob et al.[2] were the first to report the syndrome of cholinergic crisis in non-myasthenics exposed to anticholinesterase insectides, and later, to "nerve gas."[3] He classified the symptoms as muscarinic, nicotinic and central nervous system in type. Muscarinic symptoms and signs, in order of severity, include anorexia, salivation, nausea, sweating, lacrimation, blurred vision, miosis, abdominal cramping, diarrhea, tenesmus, urinary frequency, excess bronchial secretion, dyspnea, substernal pressure, pulmonary edema and incontinence of bowel and bladder. Nicotinic symptoms and signs include muscle fasciculations, "thick tongue" with slurred speech, dysphagia, muscle cramps, generalized muscle spasms and weakness. Central nervous system symptoms and signs include restlessness, anxiety, giddiness, vertigo, headache, confusion, stupor, coma and convulsions.

Neostigmine overdosage may produce practically all of these symptoms. However, when it occurs suddenly muscarinic symptoms and even fasciculations may be absent, and paralysis of muscles of deglutition and respiration may come on rapidly. This may resemble myasthenic crisis so closely that the desperate patient or physician may add even more neostigmine, with disastrous results. Cholinergic crisis is a greater threat to myasthenics who have especially weak pharyngeal and respiratory muscles, and to severe myasthenics with a fluctuating course who might fail to match a sudden remission with a rapid decrease in dosage.

In our series there are 186 severe or moderately severe myasthenics. This is the type of case in which crisis may be expected. Of these, from 1942 to December, 1954, twenty (10.8 per cent, thirteen females and seven males) have experienced crises, from which sixteen (8.6 per cent) failed to recover. Four have survived, three of them within the past two years. Duration of myasthenia before crisis ranged from six months to fifteen years, averaging 3.12 years. Onset of symptoms in practically all cases was rapid and severe, and in many the course of the disease was fulminating. All patients were receiving neostigmine at the time of crisis except one who was taking mestinon and the last three who were receiving WIN 8077.

Of the sixteen fatal cases, eight died suddenly. The remaining eight were treated in respirators from eighteen hours to twenty-one days. Two of these recovered from one crisis, only to die from a later one. Postmortem examinations were obtained in seven cases. In four, involuted thymuses were found. Two had small benign thymomas, one had an "involuted thymus with hyperplastic nodule." In only two were lymphorrhages found. In retrospect, it seems apparent that more than half of these fatal crises were of a cholinergic nature.

Of the four patients who recovered, three were treated in respirators; the fourth required only tracheotomy and suction. One of these patients was certainly in cholinergic crisis; in two this condition was probable. Myasthenic crisis developed in the fourth induced by 16 mg. of curare given at surgery to a previously undiagnosed myasthenic.

One of these patients, a twenty-four year old pregnant white woman, was hospitalized on December 8, 1952, for delivery. Her oral neostigmine dosage had risen to 215 tablets (3,225 mg.) daily. During an eight-hour labor she was given 4 mg. per hour parenterally. She was delivered of a vigorous 8 pound boy by mid-forceps. Thirty-six hours later he became obviously myasthenic. For ten days he required neostigmine but has not needed it since. Two days after delivery the mother suddenly stopped breathing. She became unconscious and deeply cyanotic but her pupils were miotic and her pulse was 80. Tracheal intubation was difficult as her jaws were powerfully clenched. She was placed in a respirator and given atropine, 1.2 mg. intravenously, and oxygen, with improvement. No neostigmine was given for twenty-four hours, during which time she continued to improve. Then she began to show increasing weakness which was relieved by small doses of neostigmine. She required the respirator intermittently for fifteen days. Since then her oral neostigmine dosage has varied between 50 and 100 tablets (750 to 1,500 mg.) daily. It would seem that this patient had a sudden partial remission after delivery but that her dosage was not reduced accordingly, thus precipitating a typical cholinergic crisis.

TREATMENT OF CRISIS

Whenever a myasthenic fails to clear secretions or to breathe adequately tracheotomy or tracheal intubation followed by tracheotomy should be performed. This insures an airway through which oxygen can be given and secretions can be aspirated. A respirator should be used without hesitation should voluntary breathing seem inadequate.

At this point an effort should be made to determine whether crisis is on a myasthenic or cholinergic basis. If excess secretions and/or miosis or relatively slow pulse are found, atropine 1.2 mg., should be given intravenously and repeated as long as these conditions are present. Although atropine will not relieve cholinergic weakness, it will alleviate most of the muscarinic symptoms. If no improvement occurs with atropine, tensilon® should be given according to the method of Osserman.[4] His most recent modification[5] consists of administration of 0.2 cc. (2 mg.) intravenously with the needle left in place. If improvement occurs, the crisis is probably myasthenic and the patient will undoubtedly respond to heavier dosage of cholinergic drugs. However, if muscle fasciculation appears, the crisis is probably cholinergic. Should no effect occur within thirty seconds, another 0.8 cc. is given. If muscle fasciculation and/or no improvement follow, cholinergic weakness should be suspected.

The course of one of our patients, a thirty-two year old Negro man who eventually died, illustrates the value of tensilon in differentiating myasthenic from cholinergic crisis. Two years previously he had survived a crisis. A year later another occurred, followed by frequent crises and intermittent psychotic episodes for seven months in which, without tensilon, it would probably have been impossible to keep him alive. During this time respirator care was repeatedly necessary. Because of his mental condition he was never able to differentiate underdosage from overdosage. We were able to do this with tensilon and his therapy was regulated accordingly, with reasonable accuracy.

Grob[2,3] has emphasized that in cholinergic crisis atropine tolerance and need is tremendous, and doses up to 2 mg. may and should be given intramuscularly at hourly intervals until signs of atropinization occur. He further advises,[8] and we agree, that cholinergic medication should be stopped until improvement occurs, followed by a return of weakness, at which time it should be cautiously resumed.

While the patient is in the respirator nothing should be given by mouth. Fluid and food requirements should be met by intravenous feedings, with a wary eye to fluid and electrolyte balance. However, as soon as the respirator is not constantly necessary, tube feeding may be used. Small amounts should be given frequently, and aspiration should be done before each feeding to guard against gastric retention. Antibiotic therapy should be started immediately and continued as long as a respirator is necessary, or as long as the patient coughs ineffectively. Should signs of atelectasis or any bronchial obstruction appear, bronchoscopy may prove to be life saving. Aranow[9] describes excellent results using mechanical exsufflation with negative pressure to prevent these conditions. He also warns against overventilation in the respirator and advises that CO_2 tension be checked frequently to avoid this hazard.

Cholinergic therapy should vary with the needs of the patient, and may be guided by

tensilon tests. Should any doubt exist as to adequacy of dosage, it is far better to underdose than to overdose. Mestinon® would seem to be the drug of choice for respirator patients because it produces less stimulation of flow of secretions and gastrointestinal activity. When cholinergic drug dosage requirements have stabilized, they may be given with intravenous fluids by continuous drip, as first recommended by Viets.[6] Atropine may be given as necessary to control excess secretions, but too much drying should be avoided. Morphine should not be given since cholinergic drugs potentiate its action.[3] Demerol® and barbiturates seem well tolerated, however, and are useful in allaying the extreme discomfort and anxiety present in crisis.

PREVENTION OF CRISES

In such a variable condition as myasthenia, rigid dosage schedules can never give adequate control. In no condition is it more important to teach each patient to vary dosage according to needs.

Patients should be taught to recognize cholinergic side effects and should be given atropine or belladonna to combat them should they occur. However, as emphasized by Schwab,[7] no severe myasthenic should ever take regular doses of atropine or belladonna to anticipate or prevent side effects without full knowledge of the danger of such a procedure. These drugs obscure or prevent such muscarinic symptoms as abdominal cramping, diarrhea, salivation and miosis. These are valuable signs of cholinergic overdosage; and, if present, warn the patient against taking more medication and thus precipitating cholinergic crisis. Unfortunately, muscarinic symptoms do not always appear before the onset of cholinergic crisis. However, patients who have experienced cholinergic weakness describe it as a peculiar "quivery" sensation, often accompanied by unusual dysphagia and dyspnea. Some patients also experience cramping or "tightness" of muscles.[8]

Because infection often precipitates crises, severe myasthenics should be supplied with an effective oral antibiotic for use at the first sign of infection.

In conclusion, it is this author's opinion that when drugs with more anticurare and less anticholinesterase activity become available, crises, both myasthenic and cholinergic, will become rare or non-existent in the treatment of myasthenia gravis.

SUMMARY

1. Crisis in myasthenia gravis is of two main types: True myasthenic crisis due to insufficient medication or drug resistance; and cholinergic crisis due to excess of cholinergic drugs.

2. Cholinergic crisis is a greater threat to myasthenics with especially weak pharyngeal and respiratory muscles, and to severe cases with a fluctuating course.

3. Differential diagnosis, treatment and prevention of crisis in myasthenia gravis are briefly presented.

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End of Symposium on Myasthenia Gravis

Clinical Studies

Acute Fatty Metamorphosis of the Liver Associated with Pregnancy*

A Distinctive Lesion

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ACUTE yellow atrophy of the liver is a literal translation of the German term, *akute gelbe Leberatrophie*, and it is construed as implying an illness of short duration and fatal outcome. The signs and symptoms of liver failure dominate the clinical picture, and jaundice is almost invariably present. The liver is usually found at autopsy to be smaller than average, soft, yellow, and to show microscopic evidence of acute lesions. Clinical entities such as subacute yellow atrophy and healed acute yellow atrophy have also been described; the implications of the terms in regard to the clinical features and the pathologic observations associated with them are obvious. Although one customarily associates acute yellow atrophy with fulminant epidemic viral hepatitis, this is not the only etiologic background for the development of the sequence of clinical events and anatomic findings. Similar phenomena have been noted in homologous serum jaundice (presumably caused by a virus somewhat different from that causing epidemic hepatitis), acute intoxications from arsenic, phosphorus, chloroform and carbon tetrachloride, Weil's disease (leptospiral hepatitis), and certain malnutritional conditions upon which some other additional (and unknown) factor is superimposed, such as kwashiorkor, etc. Certain experimentally induced dietary deficiencies produce a pathologic picture not dissimilar to that seen in kwashiorkor. Formerly, acute yellow atrophy of the liver used to be listed among the toxemias of pregnancy until it was recognized that most of such cases were merely fulminant epidemic hepatitis complicating pregnancy, and that the liver of pregnant women is damaged more readily and with greater severity by viral hepatitis than that of non-pregnant women. Frucht and Metcalfe[1] found that infectious hepatitis carried a higher mortality and a greater tendency to produce chronic liver damage when it occurred in pregnancy than when it occurred in non-pregnant women. Dill[2] reviewed the relevant literature and presented six fatal cases of acute yellow atrophy during pregnancy. He concluded that they were identical with severe infectious hepatitis. Implicit in his argument is the idea that acute yellow atrophy is an entity regularly equatable with severe forms of infectious hepatitis rather than a type of liver lesion which may develop following a variety of pathogenetic sequences. It is of some importance to note that it is generally possible to distinguish nosologically among these various etiologies of acute yellow atrophy by careful evaluation of the morbid anatomy of the liver. To this group of etiologic backgrounds may be added the "hepatorenal syndrome," an ill defined clinical entity in which death ensues after a short illness characterized by rapidly developing hepatic and renal decompensation. This syndrome embraces a wide variety of clinical phenomena and under its label is included a congeries of equivocal histopathologic changes.

In 1940 Sheehan[3] described a condition under the term "obstetric acute yellow atrophy" which he distinguished on histopathologic grounds from the more usual varieties of acute yellow atrophy. He was able to collect six cases of 400 autopsies on file at an obstetric hospital. Although he did not describe each case indi-

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vidually, his summation of the clinical and pathologic features is worth quoting in part. He states "Clinically the condition was very suggestive of true acute yellow atrophy. The usual course of the disease was as follows. At 36 to 40 weeks there was a sudden onset of severe vomiting and epigastric pain, followed in a few days by jaundice. These symptoms progressed very rapidly, the jaundice becoming very intense, the vomit was of coffee-grounds appearance, and headache was sometimes present. After 7–12 days the patient was delivered of a still-born foetus, became comatose, and died within three days after delivery. . . . At autopsy . . . the liver was yellow and rather small (1,040 to 1,300 grammes). Its lobular pattern was not lost. Microscopically all the cases showed an identical lesion: there was a gross fatty change affecting the entire lobule except a sharply defined rim of normal cells about the portal tracts. The affected cells were bloated by a fine foam of tiny fatty vacuoles throughout the cytoplasm, so that they resembled the cells of the suprarenal cortex. The nuclei were normal, and there was an entire absence of necrobiotic change . . . round cells throughout the fatty area. . . . The kidneys did not show any microscopic lesions, and in the two cases in which Sudan-stained sections were available, there was not any fatty change in the first convoluted segment."

Sheehan pointed out the paucity of reported cases in which this lesion was present prior to his publication. He cited the two cases described by Cullinan[4] in the appendix to his series of cases of "subacute necrosis of the liver" and the case reported in some detail by Stander and Cadden[5] as the only instances in which there was adequate documentation by pathologic examination of the presence of this distinctive lesion. Sheehan stressed the absence of necrosis as the most significant observation in his microscopic examination; whereas Stander and Cadden had indicated that they interpreted the fatty changes in the liver cells as a stage preceding necrosis, Sheehan pointed out that necrosis of parenchymatous organs which occurs as a result of slowly acting toxins is quite manifest histologically within two or three days, and that the patients with "obstetric acute yellow atrophy" survived for ten to fourteen days, exhibiting neither necrosis of liver cells nor any histologic indication that necrosis might have developed if they had lived longer. Sheehan also stated, "The pathological change in the livers of patients who die is of a reversible kind: if any of these patients had recovered, the liver cells could have returned to normal appearance within a few days. But, if a patient recovers, the diagnosis cannot be established with certainty, in view of the numerous other possible causes for jaundice."

Stander and Cadden present the details of the clinical and laboratory findings in a typical case of "obstetric acute yellow atrophy" stating that if, in the presence of persistent vomiting, dizziness and headache occurring in a pregnant woman, especially near term, icterus and drowsiness appear, it is almost certain that severe liver damage is present and that the prognosis is grave. The laboratory findings are those associated with severe, acute liver damage, irrespective of cause, and include increased serum bilirubin, decreased blood sugar, decreased blood urea nitrogen, increased blood uric acid, increased blood amino acids and decreased urea nitrogen in the urine. In regard to the pathologic observations upon the liver at autopsy, they point out that the liver cell nuclei are well preserved and that there is no frank necrosis to accompany the intracytoplasmic accumulation of fat within the liver cells.

Subsequent to Sheehan's report, Whitacre and Fang[6] presented a case of jaundice in pregnancy in which the microscopic appearance of the liver lesions as seen in two biopsies is identical with that described by Sheehan as well as by Cullinan and by Stander and Cadden. Their patient was a thirty year old Chinese, gravida II, para I, in whom weakness and anorexia developed followed by the onset of a respiratory infection, epigastric discomfort and vomiting of a "coffee-ground" material four weeks before term. She was icteric on admission, mentally dull, drowsy but conscious. She had moderate labor pains for four hours without progress, and her drowsiness increased to stupor. A cesarean section was performed and a live baby was delivered. The patient's liver was found to be of normal appearance and size, and a biopsy was performed. On the seventh postoperative day, a fit of coughing produced dehiscence of the abdominal incision with extrusion of loops of bowel. A secondary closure was performed and at this procedure the liver was seen to be smaller than before, distinctly yellow, and another biopsy was performed. The subsequent course was stormy, being complicated by another wound dehiscence, two profuse gastrointestinal hemorrhages, and shock. However, she responded to

treatment and recovered. Her jaundice diminished gradually and disappeared three weeks following hysterotomy. Tests of liver function showed impairment for five weeks after cesarean section. During the acute phase of her jaundice she had a low blood sugar and a low blood urea nitrogen, a transient increase in blood amino acids, a moderate elevation of blood uric acid and a low urinary nitrogen. These determinations confirm those described previously by Stander and Cadden.

Histopathologic interpretations in this case rest upon the two liver biopsies. The liver tissue removed at the time of cesarean section, when the hepatic damage was presumably maximal, showed well preserved lobular architecture. The liver cells in the central three-fourths or four-fifths of each lobule were swollen and clear, containing large amounts of fat and some clumped granular material near each nucleus. The cells at the periphery of the lobules were less clear, had less distinct cell borders, and were free of fat. A small amount of yellow pigment (presumably bile) was present in some of the central clear cells as well as in an occasional von Kupffer cell. A few lymphocytes and inconspicuous polymorphonuclear leukocytes were present in the portal spaces but the bile ducts were normal. There was no evidence of cell necrosis. The second biopsy, seven days later, showed substantially the same general features as the first except that the central clear zone of fat-containing cells was smaller, occupying only one-half of the diameter of the lobule, a slightly more conspicuous infiltration of polymorphonuclear leukocytes, lymphocytes and plasma cells, and the presence of mitotic figures in a zone lying between the central clear-cell area and the unaffected peripheral area. Whitacre and Fang were unwilling to accept this case as fitting into the group described by Sheehan, stating, "Sheehan reported 6 fatal cases very similar, if not identical, to this one as a separate entity apart from true acute yellow atrophy and suggested the name 'obstetric acute yellow atrophy.' We are not willing to do this on the basis of present knowledge, because the clinical course is identical, and the location and general type of lesion are the same. We may have to change our conception, but at this time it seems to be a matter of degree; that is, acute fatty degeneration of the liver is one stage in the process of necrosis. It seems reasonable to conclude that a dose of hormone, toxin, poison, infection, or combination of these factors could be insufficient to cause actual necrosis but still be sufficient to be usually fatal."

Of the six cases reported by Dill,[2] Case I (previously reported by Stander and Cadden) can be equated histologically with the cases described by Sheehan,[3] and Cases IV and VI present a comparable picture but cannot be assessed accurately because of the lack of a detailed report of microscopic observations.

It is of some value to note that LaDue, Schenken and Kuker[7] reported a case of phosphorus poisoning with recovery in which repeated aspiration liver biopsies were performed. The patient, a sixty-one year old white man, had swallowed an ounce of roach paste containing 2 per cent phosphorus and promptly developed signs and symptoms of acute intoxication with severe jaundice and other signs of severe liver damage. Aspiration biopsies were performed as late as the thirty-third, forty-fifth, eighty-sixth and 191st days after ingestion. Even as late as the thirty-third day areas of focal necrosis were present, and accumulation of fat in the form of both fine vacuoles and a clear type of cytoplasm were present in decreasing quantity as long as six months later. The earliest stages of healing by fibrosis were first seen in the biopsy at forty-five days after ingestion.

There is one other reported case which appears to conform in some respects to the group of cases isolated by Sheehan. Nixon, Egeli, Laqueur and Yahya[8] studied a group of twenty-one pregnant women, twelve with jaundice, in whom aspiration biopsies of the liver were performed. Their Case No. 20, a thirty-five year old primipara who developed pre-eclampsia at thirty-six weeks and gave birth twelve days later after medical treatment had rendered her normotensive and non-albuminuric, developed jaundice on the fourth postpartum day. A liver biopsy was performed at this time. Marked edema of the legs, hypertension and slight albuminuria developed the next day and she had three convulsions. On the sixth postpartum day she died with hyperpyrexia. At autopsy the liver weighed 860 gm., was of normal consistency and free from hemorrhage; the cut surface was tan and showed "small lighter stained areas evenly distributed." Microscopic examination of both the aspiration biopsy and the liver postmortem showed intact liver cell trabeculae and no necrosis. The liver lobules contained cells of two types: large polygonal cells with a clear

cytoplasm and regular, mainly vesicular nuclei, and large irregularly shaped cells with small nuclei and protoplasm very light and full of small vacuoles. Presumably, these cells contained fat and correspond to the cells described by Sheehan and the other authors cited. The presence of the antecedent unprovoked toxemia followed by apparent recovery, the recurrence of hypertension, albuminuria, edema and convulsions coincident with the onset of jaundice, make this case somewhat atypical in the small series of cases reviewed, hence difficult to evaluate. Nixon et al. considered the lesions as "change which could not be explained except by some kind of acute intoxication resulting in fatty degeneration. Such changes are seen in acute phosphorus poisoning. It is possible that this case was one of sudden functional breakdown of the liver owing to the strain of this particular pregnancy."

The three cases which serve as the basis for this article will illustrate certain of the clinical features of the course of this disease and are designed to serve as a point of departure for a discussion of certain of the pathologico-anatomic considerations which underlie an attempt to evaluate the disease process. Inevitably, certain questions will be asked which cannot be satisfactorily answered. It is hoped that such interrogations and speculations as are made will serve as a stimulus for further investigation and elucidation.

CASE REPORTS

CASE I. Mrs. E. B. (C.C.M.H. No. 34499), a twenty-four year old white, gravida II, para I, E.D.C. (expected date of confinement) about July 1, 1944, was admitted on May 14, 1944, because of nausea and vomiting of about two weeks' duration. These symptoms had been present over the entire period and had become more severe during the few days before admission. She noted that she had become jaundiced and estimated the onset of a yellowish tint to her skin as of about nine or ten days' duration. Jaundice had been slight at first but had become progressively more intense, and generalized pruritus had developed. These symptoms were accompanied by diffuse, poorly localized abdominal pain which was constant but not intense. The patient had noted that her stools had become extremely light, almost clay-colored, and that her urine had become very dark. Repeated questioning of the patient and her husband failed to elicit a history of poor diet, alcohol intake or exposure to possible liver toxins.

Physical examination revealed generalized icterus and dehydration. The abdomen was enlarged to a size consistent with a seven and one-half months' pregnancy, with the fetus in L.O.A. position. Soft distention and diffuse tenderness, more marked in the right upper abdominal quadrant, were observed. The liver edge and spleen were not palpable. There were no ecchymoses or purpuric manifestations. There was no suprapubic pain or tenderness.

On admission her temperature was 98°F., pulse 96, respirations 20, blood pressure 118/74. The admission urine specimen had a specific gravity of 1.017, was dark amber, acid, free from sugar or acetone, contained bile and a trace of albumin. The hemoglobin was 88 per cent, red blood count 4.73 million, white blood count 19,500 with 87 per cent polymorphonuclear leukocytes, 12 per cent lymphocytes and 1 per cent monocytes. A rare normoblast was found. The icterus index was 100. Examination of her vomitus was positive for occult blood and bile. The patient was treated with intravenous fluids, vitamin B complex, vitamin K, and given a high carbohydrate, high protein diet.

The day following admission she went into spontaneous labor and was delivered rapidly, almost precipitately of a 5 pound, 15 ounce girl. The membranes ruptured during the second stage of labor. No lacerations were incurred at delivery. A moderate amount of postpartum bleeding occurred, which ceased after injection of 3 ampuls of ergotrate.®

The day following delivery the hemoglobin was 62 per cent, red blood count 3.13 million, white blood count 32,000 with 77 per cent polymorphonuclear leukocytes, 5 per cent lymphocytes and 2 per cent monocytes, and a few nucleated red cells were found. Again the vomitus was positive for occult blood and bile. The same day the urine was still dark amber and acid, had a specific gravity of 1.020 was free from sugar or acetone, but was positive for bile, and had a trace of albumin. Following delivery her condition became worse. Vomiting was more severe and jaundice increased. She was treated with transfusions of compatible blood, Wangensteen suction, intravenous glucose solutions, as well as supplementary vitamin B complex and vitamin K. She became stuporous and irrational. Her temperature rose to 105.4°F., pulse 150, respira-

tions 40 and shallow, and she died on the fourth postpartum day.

At birth, the infant appeared to be in good condition but respiratory difficulties soon supervened necessitating vigorous treatment for what was diagnosed as "atelectasis." Although the infant recovered from this episode and is now living (age ten), she exhibits signs of mental retardation and emotional disturbance.

Gross observations (B.L-i.H. No. A-44-23): The postmortem examination was limited to the contents of the abdomen and pelvis. The most significant findings were in the liver and kidneys. The uterus was compatible in size and configuration with a four days' postpartum appearance, and there was no failure of the placental site to heal. Between 3,000 and 4,000 cc. of bile-stained fluid were present in the peritoneal cavity. There was moderate to marked edema of the duodenum and jejunum. The retroperitoneal tissues were also edematous and the lymph nodes in this area were enlarged, soft and juicy. The gallbladder was not thickened and contained no calculi; bile was easily expressed into the duodenum along the biliary tree, and dissection of the biliary tree revealed no obstruction. The ampulla of Vater was located in the third part of the duodenum rather than the second. The liver was not remarkable in shape but no weight is recorded. However, the liver edge was rounded. The capsule was thin, translucent and tense. On external inspection the liver markings were moderately distinct and the color varied from reddish gray to putty-colored. The cut surface showed increased prominence of the liver markings, and was pale greenish brown with a yellowish cast. No gross evidence of intrahepatic biliary obstruction was found. The bile in the smaller biliary ducts was dark, concentrated and mucoid.

The kidneys weighed approximately 110 gm. apiece. The capsule was somewhat thicker than usual but stripped with ease revealing a peculiar, congested, somewhat icteric capsular surface. Sagittal sections showed the usual gross renal architecture. The cortex was strikingly pallid and measured 7 mm. The medullary markings and striations were not remarkable. The mucosa of the pelvis was congested and the pelvis contained a small amount of turbid, icteric urine.

Microscopic observations: Sections of the liver stained with hematoxylin and eosin showed that the lobular architecture was well preserved insofar as the spatial relationship of central veins to portal areas was concerned but the individual lobules were swollen, compressing the interlobular boundaries so that the zone between lobules was indistinct. With the exception of a discontinuous thin rim of apparently normal liver cells at the periphery of the lobule, the entire lobule was composed of swollen vacuolated liver cells. (Fig. 1A.) The parenchymal cells were arranged in the usual trabecular pattern, and the relationship of liver cords to sinusoids was preserved. The conspicuous change was in the cytoplasm of these cells; almost every cell contained a myriad of fine vacuoles which were separated from each other by delicate eosinophilic cytoplasmic strands. (Fig. 1B.) Coalescence of vacuoles was not a prominent feature, and large single vacuoles pushing the nucleus to one side, producing a signet ring appearance, were not seen. The nucleus was almost invariably in the center of the cell. The nuclei appeared slightly smaller than average but this may have been more apparent than real because of the increased cytoplasmic area. The nuclei were well preserved, with a distinct chromatin pattern, were not pyknotic or fragmented, and appeared viable in every respect. Binucleated liver cells were found in quantity. The von Kupffer cells were not increased in number; a few of them exhibited a yellowish tint to their cytoplasm, presumably due to accumulation of intracytoplasmic bile pigments. Here and there a few small sprinklings of infiltrating inflammatory cells were present. For the most part these were lymphocytes but an occasional plasma cell was present among them, and a rare polymorphonuclear leukocyte could also be seen. This minimal infiltrate was not localized to any portion of the lobule; it was no more prominent in the portal spaces than in its irregular distribution in the sinusoidal spaces. Conspicuously absent was any degree whatsoever of necrosis of liver cells. Reticulum stains showed an intact fibrillar architecture to the lobules, confirming the impression gained from study of hematoxylin and eosin sections. A Hotchkiss-McManus stain failed to demonstrate mucopolysaccharides and glycoproteins within the cytoplasm of the altered liver cells. It is inferred from the size, shape and transparency of the vacuoles that they represent intracytoplasmic lipid within the cells.

The general architecture of the kidney was well preserved. The glomeruli were somewhat more plump than usual, and there was some prominence of the capillary loops as well as

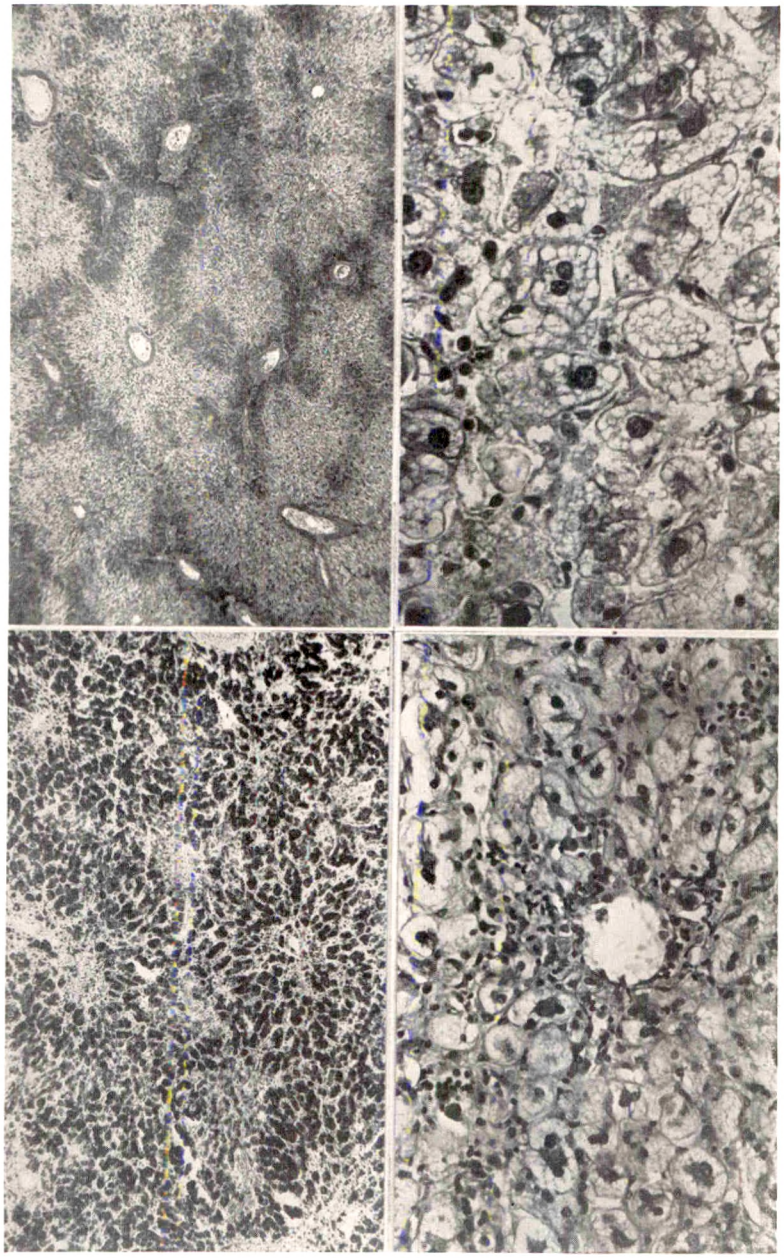


FIG. 1. A, Case I. Low power view showing pale areas of fatty metamorphosis in the central two-thirds of the lobule surrounded by a darker rim of spared liver cells; hematoxylin and eosin stain, liver. B, Case I. Detail of liver cells with fatty metamorphosis showing multiple fine vacuoles distending the cytoplasm of the cells. A binucleate cell is shown; hematoxylin and eosin stain, liver. C, Case II. Low power view showing distribution of fat in the liver lobules; oil red O stain, liver. D, Case II. Detail showing central vein with slight paracentral necrosis and a surrounding zone of fat-filled cells with multiple tiny vacuoles honeycombing the cytoplasm; hematoxylin and eosin stain, liver.

equivocal increase in the amount of the glomerular interstitial substance. However, the most striking changes were in those tubules which lay within the medullary rays of Ferrein. The epithelium lining these tubules was pallid, and the cytoplasm of the epithelial cells finely foamy or vacuolated. (Figs. 2B, C.) The change was sharply localized to this area for it was not present in the upper portion of the proximal or in the distal convoluted segments which lay elsewhere in the cortex. The nuclei of cells so affected were indistinct and their chromatin stained poorly; whether or not this is true necrobiosis is difficult to state with certainty. No lesions were seen in the renal vasculature and the medulla was unremarkable. Although formalin-fixed tissue was not available for Sudan stain, the alteration was interpreted as compatible with fatty accumulation within the cytoplasm.

The pancreas was histologically normal, apart from slight interstitial edema. No necrosis was noted.

The final anatomic diagnoses were fatty metamorphosis, liver, acute, massive; fatty metamorphosis, kidneys, medullary rays of Ferrein; ascites; lymphadenitis, retroperitoneal nodes; icterus, generalized; status postpartum, uterus.

Case II. Mrs. T. C. (M. H. No. B3243),* a twenty-six year old primipara due to deliver on October 20, 1952, was admitted to the Milton Hospital on October 8th. She had had a completely uneventful prenatal course with no sign of elevated blood pressure or other difficulty. Previous health had been excellent. No history of dietary deficiency or of exposure to liver poisons was elicited at this time or on subsequent questioning of her husband. She was group O, Rh positive. On October 6th she complained of severe headache, with no other signs. Her blood pressure was then 120/80. The urine was negative. She was given codeine and empirin without relief. On October 7th the headache was still present and she vomited. She was then sent to the hospital where her temperature was found to be 100.6°F., pulse, 100, respirations 20, blood pressure 128/80. She was given 300,000 units of penicillin. The fetal heart rate was found to be 136. The urine was reported negative. On October 9th the temperature was normal but the white blood count was 17,000 with three nucleated red cells. The mother's heart rate was now 120. The fetal heart was not heard, possibly due to the loud action of the mother's heart. A lumbar puncture was done which yielded clear fluid with no cells. On October 10th the patient was semi-comatose but was rational when roused. The blood pressure was 120/80. The pulse was 100. The urine now showed a 1+ albumin and fairly numerous hyaline and granular casts, as well as ten to twelve white blood cells per high power field. The leukocyte count in the blood was 20,400. The uric acid was 5.6 mg. per cent. The serum NPN was 74 mg. per cent. On the following day she was found to be definitely jaundiced. She was in active labor in the morning and was semi-comatose, with blood pressure of 120/80. She was delivered by low forceps of a dead female fetus which was not macerated but showed a soft skull. After delivery there was persistent oozing and it was found necessary to pack the uterus. Coma deepened and several hours later the patient died. The bilirubin was found to be 6.2 mg. per cent total, with 3.8 mg. per cent direct. The serum NPN on that day was 72 mg. per cent. The chlorides were 95 mEq./L. The thymol turbidity was four Maclagan units. Prothrombin concentration by Quick's method was 11 per cent.

Gross observations (F. H. No. U-52-32): Postmortem examination was limited to the thorax and abdomen. The body was well developed and well nourished. A thin, brown fluid trickled from the corners of the mouth. Each pleural cavity contained 2 or 3 cc. of clear fluid. The heart weighed 300 gm. and was not unusual in any way except for a small subendocardial hemorrhage in the left ventricle. The lungs weighed 340 and 310 gm., respectively, and showed a few small scattered elevated red-brown spots evidently due to terminal aspiration of brown "coffee-ground" fluid, which was observed in the mouth, stomach and bronchi. The peritoneal cavity contained about 15 cc. of bright red bloody fluid which had apparently oozed through from the parametrium. The gastrointestinal tract was remarkable only for the presence of pale, almost clay-colored feces in the colon; no ulcers were found. The only other significant findings in the abdomen involved the uterus, liver and kidneys. The uterus showed the expected postpartum appearance, with the addition of rather extensive hemorrhage into the parametrium on both sides. The liver was decidedly shrunken, weighing only 810 gm. The capsule was thin and

* The authors are grateful to Dr. Augustine Rogers for permission to present the clinical history of this case.

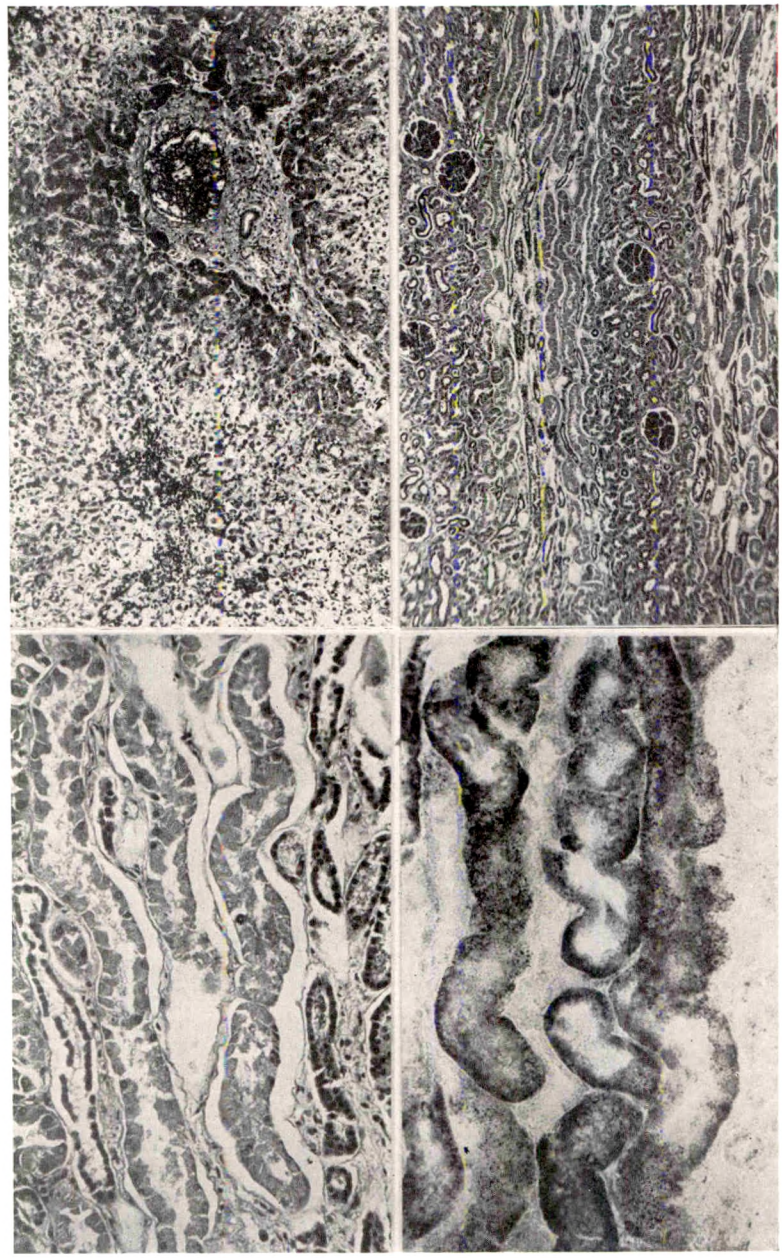


FIG. 2. A, Case II. Detail showing the peripheral spared area near the portal space in contrast with the central fat-filled area of the lobule; Gomori trichrome stain, liver. B, Case I. Low power view showing pale staining of the columns of Ferrein contrasting with more intense staining of the convoluted segments; hematoxylin and eosin stain, kidney. C, Case I. Detail of tubules in the column of Ferrein showing pale staining interpreted as fine fatty droplets; hematoxylin and eosin stain, kidney. D, Case II. Detail of proximal convoluted tubules showing fine fatty droplets within the cytoplasm of epithelial cells; oil red O stain, kidney.

smooth but had a tendency to wrinkle. Externally the liver was flabby and had a dull yellow-pink color. On section the tissue seemed more friable than usual, and the color was a dull pale yellow with numerous scattered minute red spots corresponding to the lobular markings. There was no difference between right and left lobes, and there was no obstruction of the intra- or extrahepatic vessels or bile ducts. The gallbladder was small, containing 3 or 4 cc. of viscid green bile with no calculi. The common bile duct was not dilated. The kidneys weighed 130 and 160 gm., respectively. They were unusually pale, probably because of blood loss. The capsules stripped easily from each other, revealing a pale yellow-pink surface which was smooth except for a few scattered shallow depressions measuring from 0.2 to 0.8 cm. in diameter. On section the cut surface showed a slightly swollen appearance with a yellowish cast on a pinkish tan background, but cortex and medulla were well defined, and some of the cortical striations could be seen. The pelves, calyces and ureters were not dilated and were lined by an intact pale mucosa except for a few petechial hemorrhages in one renal pelvis. The placenta weighed 450 gm. and was not remarkable. Permission for fetal autopsy was not granted.

Microscopic observations: Aside from a moderate chronic gastritis, significant findings were confined to the liver and kidneys.

The liver closely resembled that of Case I in that all the hepatic cells, apart from a narrow well preserved zone around the portal areas, were filled with cytoplasmic vacuoles, several to each cell. (Fig. 2A.) Fat stains confirmed the lipid nature of these vacuoles and that several were present in each cell, in contrast to the single, signet ring appearance often seen in fatty livers. (Fig. 1C.) Although the affected cells were clearly swollen and larger than usual, and no frank necrosis was seen, it was obvious that the portal zones and central veins were closer to one another than they are in the normal liver. This implies a decrease in site of the lobules due to loss of liver cells, as indeed must have been the case in view of the small size of the liver. Perhaps to be correlated with this fact was the observation of groups of cells containing fine yellow pigment granules, situated near the central veins, usually in the company of a few lymphocytes and occasional polymorphonuclear leukocytes. (Fig. 1D.) These groups of cells appeared to lie within spaces which at first sight appeared to be the usual blood sinusoids. However, erythrocytes were usually not present and an endothelial lining was not often demonstrable. While many of the pigmented cells had reniform nuclei, others had round or oval nuclei, and it is conceivable that some of them might be bile-stained dying liver cells, although certainly many are phagocytes. Another finding, not prominent in Case I, was an abundance of bile "plugs" in the canaliculi of the central and mid-portions of the lobules. Thus an obstructive element is added in this instance.

The kidney showed a few small subcortical scars, associated with rather heavy infiltration of lymphocytes and apparently representing a healed focal pyelonephritis. Otherwise the renal parenchyma, in the hematoxylin-eosin stain, was not remarkable except for a few vaguely defined vacuoles in the proximal convoluted tubules and a little granular precipitate in the lumina of these tubules. The fat stain, however, showed an abundance of fat droplets in the proximal convoluted tubules and in the collecting tubules of the medulla, but not in the distal convoluted tubules. The fat in the proximal tubules was arranged in relatively small but numerous globules in the basal parts of the cells. (Fig. 2D.)

The placenta was not remarkable. It showed fairly numerous foci of calcification in the intervillous fibrin deposits but no necrosis or vascular lesions.

The final anatomic diagnoses were fatty metamorphosis, liver, massive, acute; fatty metamorphosis, renal tubular epithelium; aspiration of gastric contents, agonal; status postpartum, uterus.

CASE III. Miss G. B. (F. C. H. No. 1443),* a sixteen year old primigravida, was admitted to the Florence Crittenton Hospital on August 8, 1928, at seven and one-half months' gestation. Her last menstrual period had been on December 20, 1927, and her E.D.C. was September 27, 1928. She was in good health when admitted and remained so until September 8th when she began to vomit. She was sent to bed and was given an enema on the following day. Her vomiting ceased but she spent both September 9th and 10th in bed, acted queerly, talked

* This case is reported through the courtesy of Dr. G. Kenneth Mallory, who recalled having performed the autopsy when the initial manuscript was given him. Dr. Mallory graciously placed this case at the authors' disposal and prepared additional sections for study.

irrationally and became somewhat unresponsive. At 4:00 A.M. on September 11th she was found sitting on the floor counting aloud. She was put back to bed, making no resistance and went to sleep. At daylight she was noted to be jaundiced and quite irrational. At 8:20 A.M. her membranes ruptured spontaneously and she went into good labor promptly. A slightly macerated stillborn male infant weighing 6 pounds was delivered at 10:50 A.M. and the placenta at 11:45 A.M. The placenta was small, yellowish and hard. While repairing a small perineal tear, it was noted that the patient was in poor condition with a pulse of 120. She was given a subcutaneous saline infusion, stimulants, etc., but failed rapidly and died at 12:05 P.M. No urine had been obtained by catheter during delivery.

Gross observations (Mallory Institute of Pathology No. U-28-36): The body was that of a well developed and well nourished girl, 157 cm. long. The skin and scleras were icteric. Slight rigor mortis but no lividity or edema was present. The liver was smaller than average (not weighed), and extended 5 cm. below the xiphoid and 2 cm. below the costal margin in the midclavicular line. The external surface was smooth and pale yellow with superficial capillary injection. The substance of the liver was firm and the cut surface was homogeneous and yellow. There was no evidence of obstruction in the gallbladder or bile ducts. The kidneys were normal in size and pale yellow brown. The capsule peeled easily revealing a smooth surface. The cut surface showed an even cortex 0.5 cm. thick. The renal pelves showed no lesions. The uterus was compatible in appearance with a recent postpartum state. The other viscera were grossly unremarkable.

Microscopic observations: As in the two previously cited cases, the pathologic changes were confined to the liver and kidneys. Sections stained with phloxine-methylene blue showed that the lobular architecture of the liver was well preserved and that no necrosis was present. The liver trabeculae were composed of well preserved cells with extensive vacuolation of their cytoplasm, the vacuoles being rather small, many being present in each cell, producing a coarsely foamy appearance. The nuclei of these cells were centrally located and their chromatin was distinct and well preserved. Occasional binucleate cells were seen. This vacuolation occupied almost the entire thickness of each lobule, there being only a thin rim of spared liver cells adjacent to each portal space. The vacuolated cells were ballooned and the sinusoids compressed. There was no evidence of bile stasis but occasionally bile pigment was seen in von Kupffer cells. A scattered and inconspicuous infiltration of lymphocytes and an infrequent plasma cell were found sporadically in portal spaces and occasionally in lobules. Formalin-fixed tissue was not available for fat stains.

The kidneys showed the usual microscopic architecture. The glomeruli were well preserved and free from infiltration or scarring. Examination of the epithelium of the proximal convoluted segment revealed the cytoplasm of many cells to contain small vacuoles. The nuclei of these cells were well preserved and stained sharply. In the medullary rays of Ferrein the tubular epithelium became pale and eosinophilic, and nuclear staining was indistinct. However, in these cells also there was fine vacuolation of the cytoplasm. Although tissue was not available for fat stain, this was interpreted (as in Case 1) as a combination of fatty metamorphosis and postmortem autolysis. No changes were seen in the blood vessels or interstitial tissue of the kidneys.

The final anatomic diagnoses were fatty metamorphosis, liver, acute, massive; fatty metamorphosis, renal tubular epithelium; status postpartum, uterus.

COMMENTS

The clinical aspects of these cases seem to be reasonably well defined. The clinical course is characterized by the onset of jaundice late in the third trimester of pregnancy. Hemolytic and obstructive jaundice can be ruled out. The disease is clearly one of the varieties of parenchymatous (hepatocellular) jaundice. The signs, symptoms and laboratory in disations of acute liver failure develop. The patients become progressively worse even after delivery and die in hepatic failure. On purely clinical grounds the disease is not distinguishable from fulminant epidemic hepatitis. Of the fourteen cases reported (including the present three), thirteen cases have been fatal, a mortality rate of 92.9 per cent. Only the case reported by Whitacre and Fang recovered. Whether this is an isolated instance or whether a number of the cases diagnosed clinically as "acute yellow atrophy" but not confirmed by autopsy were in reality this type of lesion, or whether the disease does occur in a less fulminant form in which there is

recovery (with or without residual fibrosis) are matters for speculation. It is doubtful whether the diagnosis can be made antemortem without morphologic confirmation from a liver biopsy.

Likewise, the pathologic findings in these cases are reasonably consistent. There is diffuse fatty metamorphosis of the liver with preservation of lobular architecture. The peripheral portion of the lobule is spared. There is either no evident necrosis or merely minimal paracentral necrosis, albeit there is evidence of loss of cells in Case II. There is either no infiltration by inflammatory cells or else a sparse, randomly distributed infiltration of a few lymphocytes, an occasional polymorphonuclear leukocyte and a rare eosinophil. There may or may not be fatty metamorphosis of the renal tubular epithelium. On purely histopathologic criteria, the lesion in these cases cannot be classed as infectious hepatitis. It resembles more closely the effects of an exotoxin or an endotoxin upon the liver.

While this manuscript was being prepared for publication, a fourth case of this clinical syndrome associated with this distinctive liver lesion was brought to our attention.* The patient, a twenty-nine year old woman, died forty-three hours after delivery of a stillborn infant, having been ill for one week prior to the onset of labor. Postmortem examination revealed an acute hemorrhagic pancreatitis in addition to the central multilocular fatty metamorphosis of the liver without necrosis. The combination of these two lesions was reminiscent of those seen in experimental ethionine intoxication.

A body of experimental evidence has recently been accumulated that ethionine, a chemical analogue of methionine which enters into metabolic competition with it, can produce necrotizing pancreatitis and fatty metamorphosis of the liver cells. Goldberg, Chaikoff and Dodge[9,10] noted that the pancreatic lesion was restricted to the acinar tissue and that the islets of Langerhans were spared. Farber and Popper[11] were able to prevent the appearance of the liver and pancreatic lesions by simultaneous administration of methionine, but not by cysteine or glucose (which latter does prevent the liver lesion). Stekol and Weiss[12] observed that smaller doses of ethionine inhibited the growth of mice but that this inhibition wss reversed by methionine or choline, but not by cysteine or homocysteine. Wachstein and Meisel[13] found that the effects of ethionine were enhanced by preliminary dietary protein depletion. Simpson, Farber and Tarver[14] demonstrated that ethionine affects protein synthesis by inhibiting the incorporation of methionine and glycine into protein in the liver. Bollag and Gallico[15] showed alterations in enzyme activity even before histologic changes appeared; pancreatic diastase was reduced and then abolished; pancreatic catalase was somewhat increased; liver catalase activity was conspicuously depressed. The syndrome of ethionine intoxication resembles closely kwashiorkor; Brock and Autret[16] have suggested methionine deficiency as one of the possible primary etiologic factors in the development of kwashiorkor. Jensen, Chaikoff and Tarver[17] found an increased total lipid content in the livers of fasted female rats to whom ethionine had been administered. This increase was in the fatty acid moiety, there being no increase in the cholesterol or phospholipid fractions. A control series of fasted male rats also given ethionine failed to demonstrate increased lipids.

Following the procedures of Jensen and his colleagues, chemical determinations of the lipid contents were done on formalin-fixed material from Case II. It must be noted that Jensen et al. used fresh liver, but this was not available to us, and may limit the accuracy of the quantified data. Using 5.49 gm. of formalin-fixed liver, it was determined that total lipids were 13.04 per cent, total fatty acids 10.8 per cent, phospholipids 0.23 per cent, total cholesterol 0.19 per cent, free cholesterol 0.14 per cent and cholesterol esters 0.05 per cent. This corresponds closely with the values given for hepatic lipids in ethionine-treated fasted female rats. It is clear that in both instances there is an increase in liver fat due to an increase in fatty acids.

It is of some interest that in these cases the intracellular lipid appears quite regularly as fine droplets which give a honeycombed appearance to the cytoplasm. The more usually found large, single, intracytoplasmic vacuole which pushes the nucleus off to one side is conspicuously absent in these cases. Whether this is an artefact of the rate of intracellular lipid accumulation or whether it is a specific property of fatty acids in contrast to neutral fats seems to be a matter regarding which one can merely speculate.

Another feature of this type of lesion which must perforce lead to speculation is the problem of the liver weight. Apparently, there is con-

* This case will be reported in detail by Dr. Aaron W. Newton and Dr. Harwood Cummings.

siderable range in the weight of the liver at autopsy in these cases—from 810 to 1,300 gm. In view of the fairly obvious observation that the liver cells are individually increased in size, and that there is no necrosis of liver cells, does this imply a disappearance of liver cells without trace? In fact, are the liver cells numerically reduced, that is, is this a true atrophy? Measurement of the radii of lobules and of the distances from one portal space to another fail to show decrease in the size of lobules. By inference, the fact that individual liver cells are enlarged indicates that there are fewer cells per lobule. The specific gravity of the fat-laden cells is lower than that of normal liver cells. Presumably, the decrease in liver weight can be ascribed largely to the accumulated lipid.

Parenthetically, the occasional observation of paracentral necrosis near an occasional central vein need not be construed to indicate that a necrotizing agent (viral, bacterial or chemical) is etiologically implicated. Glynn and Himsworth[18] have demonstrated that the intralobular circulation is greatly restricted during the phase of acute fatty metamorphosis because the swollen liver cells narrow the sinusoids, thereby favoring ischemic necrosis in and about the centrilobular zone.

A question that will naturally be raised is whether one of the halogenated hydrocarbons (e.g. carbon tetrachloride) might have been involved in either or both of the cases, since these agents are known to produce fatty livers and renal damage. Aside from the fact that persistent questioning of relatives revealed no exposure in either patient, it may be suggested that the lesions in both liver and kidney would be expected to be much more severe if these drugs played a role. Williams[19] points out that in moderately advanced carbon tetrachloride intoxication following the administration of a single dose to mice there is extensive central necrosis, but that in early carbon tetrachloride intoxication the changes resemble those seen in starved mice in which there is no necrosis. His detailed analysis of the cytoplasm of affected cells showed increased lipid, decreased protein, decreased glycogen, as well as increased alkaline phosphatase, decreased ribonucleic acid, arginine, tyrosine or histidine. He points out that these intracellular changes are reversible four to six days after the single injection of the exotoxin. Williams also noted that in irreversibly damaged (i.e. necrotic) liver cells a PAS-positive polysaccharide-protein complex was present. This was not found in any of the three cases herein reported and further confirms statements regarding the absence of necrosis and the reversibility of the lesion.

The renal lesions in these three cases furnish evidence that the disease process is not confined to one organ. In Case I, degenerative changes were noted to be sharply limited to the columns of Ferrein, presumably involving only the terminal portion of the proximal convoluted segment. The lesion consisted of alteration of the tubular epithelium; the cytoplasm of the cells was finely vacuolated and somewhat more eosinophilic than elsewhere in the tubular epithelium. Also, the nuclear outline was indistinct and nuclear chromatin did not stain as sharply as elsewhere. Whether these observations add up to fatty degeneration without true necrosis or whether true necrosis of the epithelial cells in this segment of the tubule was present is difficult to assess. In Case II the lesion in the tubular epithelium was less sharply localized; there was fine fatty vacuolation of many parts of the proximal convoluted segment; no necrosis was evident. There is some evidence from experimental sources that the concatenation of the liver lesions seen in these cases with changes in the renal tubules is not entirely fortuitous. Farber, Simpson and Tarver[20] noted fat in the epithelium of the convoluted tubules in fasted female rats fed ethionine. Wachstein and Meisel[21] observed that normal rats when fed ethionine would develop necrosis of tubular epithelium limited to the terminal portion of the convoluted segment; they also observed that, like the liver lesions, the renal lesions were increased in severity if the animals were placed upon a protein-depleted diet and that the lesions were prevented by administration of methionine. Wachstein and Meisel pointed out that the renal lesion was similar to that produced by administration of *d,l*-serine. This type of experimental evidence implies that in the two clinical cases described there may be some comparable process of biologic antagonism or competitive metabolism which underlies the development of the lesions. Parenthetically, both Wachstein and Meisel as well as Alvizouri and Warren,[22] who confirmed their observations, indicate that the lesion of the renal tubular epithelium is reversible in experimental animals.

The occurrence of these cases exclusively in the last trimester of pregnancy raises the inevitable

question: In what way is the development and nature of the lesion related to the pregnant state? Is there anything specific about the pregnant state which would favor the development of such a lesion? A certain amount of indirect evidence may be adduced regarding this matter. In general, the normal liver is able to function throughout pregnancy without overt signs of functional or morphologic failure. Ingerslev and Teilum[23] performed needle biopsies of the liver in healthy pregnant women and found no anatomic evidence to support the concept of "Schwangerschaftleber"; because of this observation as well as innumerable reports of normal liver function tests during pregnancy, the concept has generally been discarded. On the other hand, there are certain clinical observations which indicate a "lowered hepatic reserve" during pregnancy. Just what this "reserve" is seems difficult to define, for the essence of liver function is that there is one type of cell which apparently performs all the innumerable metabolic functions of the liver. There is, however, a quantitative limit to the ability of the liver cells to function, either as a unit or in aggregate. For example, the spider angiomas and liver palms which are frequently found in patients who suffer from liver disease are frequently seen in healthy pregnant women. The appearance of these peripheral phenomena is ascribed to the inability of the liver to metabolize estrogens at the rate at which they are being produced. The comparison of estrogen inactivation by the diseased liver in the presence of normal estrogen production or the hyperestrogenism of pregnancy leads one to the generalization that a given unit weight of liver tissue (or a given number of liver cells) can inactivate a given quantity of estrogen in a given period of time. This principle can be applied to any of the other functions of the liver cell.

Biskind[24] has summarized the relation of nutritional deficiency to inactivation of estrogen in the liver, and has shown that the ability of the liver cell to perform this one function depends upon the maintenance of adequate levels of certain vitamins. He points out that "impairment of the estrogen-inactivating mechanism of the liver occurred in the absence of detectable morphologic change in this organ; conversely, inactivation of estrogen can occur in livers which are the site of severe necrosis and fat infiltration. . . . The functional and morphologic changes in the liver thus bear no necessary relation to each other." This observation is applicable in greater or lesser degree to any individual function of the liver cell one may care to investigate. However, Himsworth and Glynn[25,26] were able to produce two separate and distinct types of liver damage in rats. By utilizing a diet low in proteins they were able to produce massive hepatic necrosis which, although often fatal to their experimental animals, did on occasion heal with the production of "postnecrotic scarring." Utilizing a diet which contained either an excess of fat or a deficiency in lipotropic factors, such as choline, they were able to produce extensive fatty infiltration of the liver as well as diffuse hepatic fibrosis. Subsequently, they demonstrated that a diet deficient in protein but containing adequate amounts of methionine would prevent the development of massive hepatic necrosis. Handler and Dubin[27] confirmed the observations that a choline deficient diet would produce fatty infiltration and diffuse fibrosis of the liver, and demonstrated that either thiamine deficiency or restricted food consumption would prevent the accumulation of excessive liver fat. Subsequently, Glynn[28] observed that "considering the nutritional priority that nature grants to the developing fetus, it is not surprising that pregnancy tends to reveal states of latent malnutrition. A dietary level of thio-amino acids barely sufficient for a non-pregnant woman would become grossly deficient in the presence of pregnancy. . . . Development of a dietetically induced yellow atrophy in pregnancy necessitates a nice balance between the amount of vitamin E necessary to maintain pregnancy and the amount required to maintain the integrity of the liver, too much preventing necrosis and too little preventing pregnancy." Presumably this applies only to the acute liver necrosis in rats which can be induced by thio-amino acid deficiency and prevented by adequate vitamin E intake. However, there are probably additional variables in the diet of pregnant women which, acting either singly or in concert, are in nice equilibrium between liver necrosis and pregnancy failure. It is not unreasonable to infer that as pregnancy progresses, as the fetus develops, and as its metabolic and nutritional needs increase, the margin between the requirements of a given nutrient and an insufficiency of that nutrient becomes progressively narrowed. As the threshold of insufficiency is approached the homeostatic mechanisms

which control the sum total of liver functions are maintained at progressively changing quantitative equilibria; when equilibrium cannot be maintained either because of decreased intake, excessive demand, or both, functional failure of the liver may ensue. This may or may not be accompanied by anatomic changes in the liver cell. Presumably, if functional failure is present to any significant degree, especially in regard to certain of the more important functions, and for any significant time, there will be some morphologic evidence that such has been the case. Put more simply, the pathologic changes may occur a little later than the functional changes, i.e., an experimental animal or a patient may have a poorly functioning liver for some time before actual lesions can be demonstrated. The integrity of the liver—both functionally and structurally—may be jeopardized for some time before it is finally compromised.

From such evidence and speculation it is apparent that the disturbance in the liver is presumably the result of a metabolic injury. We can propose two alternative mechanisms for its genesis. First, this may represent acute nutritional failure. In view of the lack of specific data regarding the dietary habits of these three patients for the period of time shortly preceding and during the early stages of their illness, nutritional failure cannot be excluded. Certainly, one cannot specify which nutrient or combination of nutrients was deficient in relation to the demand for it. Alternatively, the physiologic failure of the liver and its structural changes may conceivably be the result of the action of some humoral agent which either is present because the patient is pregnant or exerts an abnormally destructive effect in the pregnant state. Whether such a hypothetic substance might be a hormone or toxin is, again, a matter for speculation. Support for either hypothesis could be adduced from the case reported by Whitacre and Fang in which rather rapid recovery ensued following intensive therapeutic efforts at supplying the liver with various substances required for the maintenance of its structural as well as its functional integrity, *and* following evacuation of the products of conception. Whether either of these hypotheses is correct, or whether this lesion is the result of another mechanism, it is apparent that the metabolic injury to the liver is one which interrupts the conversion and mobilization of fat, possibly due either to inhibition of an enzyme system or to biologic anatagonism to a necessary metabolite, or both. We would speculate further that the ability of the liver to perform transmethylation is seriously compromised at an early stage in the development of this process. We have no evidence to inform us at which point in its development the liver lesion might become irreversible if, indeed, it ever does. Nor is there any information which would permit one to speculate whether reversal of the morphologic changes would be complete, or whether healing would or would not be accompanied by diffuse fibrosis.

There are certain therapeutic implications which must be stated, however fragmentary our knowledge of this distinctive syndrome may be. Once an antemortem diagnosis has been established—and needle biopsy of the liver seems to be the only present method of diagnosis—vigorous treatment of the patient with fat-free, protein-high intake as well as parenteral fluids and vitamins as indicated seems to be the course of prudence. The administration of choline and methionine has a justification in studies on experimental animals; while it is doubtful that they can be dangerous to human patients, their therapeutic value is still *sub judice*. The use of parenteral protein hydrolysates would appear to be optional. The question of interruption of pregnancy if the patient does not respond promptly to vigorous therapy should be considered. The high fetal mortality in these cases as well as the progressive nature of the disease suggest that, if the diagnosis and therapeutic trial are made promptly, interruption of pregnancy might be in the best interests of mother and child.

In this respect an analogy might be drawn from studies of cases of epidemic infectious hepatitis acquired during pregnancy. Apparently, the usual epidemic mortality of 5 per cent is increased to 20–30 per cent in cases acquired during the last trimester of pregnancy, presumably because of the decreased margin of hepatic reserve. Likewise, in eclampsia, also a disease of the last trimester, in which hepatic lesions are commonly found, the spontaneous or operative evacuation of the products of conception is often of therapeutic benefit. It is debatable whether the formerly designated variety of toxemia also known as acute yellow atrophy was identical with fulminant epidemic hepatitis, or with the distinctive fatty metamorphosis here described, or whether indeed, the term, acute

yellow atrophy, was applied indiscriminately to both lesions because of the difficulty in distinguishing between them clinically or the failure to use precise histopathologic criteria in distinguishing between the two lesions, in patients who received the benefit of autopsy.

SUMMARY

1. Three fatal cases of jaundice occurring at the end of the last trimester of pregnancy are presented. Clinically, the course of these patients is indistinguishable from that of fulminant epidemic (viral) hepatitis. Pathologically, *the lesion is readily distinguishable from infectious hepatitis*, and consists of extensive fatty metamorphosis of liver cells occupying the central two-thirds of the liver lobule. There is no significant infiltration of inflammatory cells nor is there evident necrosis of liver cells. These three cases are similar to those described by Sheehan as "obstetric acute yellow atrophy."

2. The relevant literature is reviewed. Eleven cases have been reported previously. Including the present three cases, only one patient has recovered, a mortality of 92.9 per cent. Presumably, the lesion is reversible.

3. The effect of pregnancy on liver function and its presumptive role in the genesis of this type of lesion is discussed. Likewise, both clinical and experimental methods of protecting the liver from fatty changes are mentioned and possible therapeutic measures are indicated.

4. A variety of experimental data pertaining to fatty changes in the liver is reviewed. These include the role of the liver in inactivation of hormones, the production of fatty livers by dietary deficiencies, by exotoxins, and by biologic antagonism of competitive metabolites (e.g., ethionine). In none of the three clinical cases presented here was there any evidence for or history of dietary deficiency or exposure to or ingestion of an exotoxin. It is inferred that the pathogenesis of this obscure and uncommon lesion lies in some endogenous metabolic aberration which, possibly, produces fatty changes in the liver by interference with enzyme systems, speculatively, those concerned with transmethylation.

5. Chemical analysis of the formalin-fixed liver in one of these cases shows that the increased lipid lies almost entirely in the fatty acid moiety.

6. Fatty metamorphosis was also present in the renal tubular epithelium in all three cases.

Acknowledgments: The authors wish to thank Dr. Charles C. Roby and Dr. George V. Mann for the chemical determinations of lipid, and Dr. G. Kenneth Mallory, Dr. Duncan E. Reid, and Dr. James Metcalfe for reading the manuscript before publication. The photomicrographs were taken by Mr. Ralph M. Glazier, Medical Illustrator of the Boston Lying-in Hospital.

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Disturbances of Impulse Formation and Conduction in the Pre-excitation (WPW) Syndrome—Their Bearing on Its Mechanism*

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SOLUTION of the riddle of ventricular pre-excitation (the Wolff-Parkinson-White (WPW) syndrome) has been attempted in various ways. The problem has been studied experimentally and at the necropsy table. Special electrocardiographic leads and vectorcardiography have been used to trace the course of the anomalous pre-excitation (delta) wave. The response of ventricular pre-excitation to extraneous influences like exercise, nerve stimulation and drugs has been recorded in the electrocardiogram and analyzed. Alterations of cardiodynamics associated with the appearance of the anomalous beats were investigated with the help of cardiac catheterization and electrokymography.

Exhaustive and excellent reviews of the voluminous literature appeared about a decade ago[1,2] and recently.[3,4] Among the many theories which have been advanced, differing in fundamental concepts or in shading, two major dissenting views have evolved, both compatible with present knowledge of cardiac physiology; in fact, both were considered in one of the original studies.[5] The first is the hypothesis of an hyperexcitable ventricular focus responding (during sinus rhythm) prematurely to the mechanical or electrical stimulus of atrial activity and leading, on occasion, to a burst of rapid heart action. In its favor has been claimed particularly the possibility of eliciting, in man as well as in the experimental animal, patterns of ventricular pre-excitation by mechanical stimulation of the ventricular septum,[4,6] e.g., in the course of cardiac catheterization,[7,8] and the observation of ventricular premature beats of pre-excitation contour.[9] However, this concept fails to account for the predominance of supraventricular tachycardia in the syndrome, nor can it explain the occurrence of pre-excitation complexes during auricular fibrillation and in association with A-V block. The other concept implies the presence of one (or more) accessory conduction pathways[10–12] bypassing the physiologic impedance to conduction in the A-V node. This view is supported by the anatomic confirmation of such anomalous A-V bridges in man in about half of the cases examined at necropsy,[1,13–19] and the successful experimental reproduction of such a bypass syndrome.[20] Moreover it has the advantage that it can account satisfactorily for the many facets of the syndrome, including the various types of ectopic impulse formation and the occurrence of pre-excitation in the presence of A-V block.

Since in this controversy of views the role of cardiac arrhythmias has received only secondary attention, it seemed worth while to concentrate upon this aspect of the pre-excitation syndrome in the hope that its evaluation in the light of the two conflicting concepts might aid in solution of the problem. The following report deals with five cases of ventricular pre-excitation selected because of some particular features of disturbance of impulse formation or conduction, all, we believe, having a definite bearing on the understanding of mechanisms involved in the pre-excitation syndrome. Figures 1 and 2 show the multiplicity of ectopic supraventricular rhythms which may be observed on different occasions in the same patient. In addition, Figure 1 illustrates the various types of ventricular response to rapid impulse formation, and Figure 2 the different responses to carotid sinus stimulation.

* From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago, Ill. Aided by the Michael Reese Research Foundation and the Hibse Heart Research Fund. Presented in part at the 2nd World Congress of Cardiology, September, 1954. Washington, D. C.

In Figure 3 is reproduced an unusual case of ventricular pre-excitation in the presence of an abnormally prolonged A-V conduction time. Figure 4 illustrates the operation of an independent parasystolic focus in pre-excitation, while in Figure 5 an ectopic focus, apparently located within an anomalous A-V connection, acts to cause single and regular runs of ectopic beats. For a detailed analysis of each case, and an evaluation with respect to the two pre-excitation theories, the reader is referred to the section on Illustrative Appendix. Our viewpoints on the mechanism of ventricular pre-excitation, arrived at on the basis of the interpretation of these five cases, of additional material available in the files of the Heart Station of the Michael Reese Hospital, and a study of the pertinent literature, are outlined subsequently. New evidence was found in the course of this study to fortify the hypothesis that an anomalous A-V connection is in operation in the pre-excitation syndrome.

The Pre-excitation Beat. The typical electrocardiographic contour of the pre-excitation complex has the characteristics of a ventricular fusion beat.[1,21,22] Its early anomalous component,[23] the delta wave,[24] indicates that in some way activation of the ventricles starts in a devious fashion, by early excitation of certain portions of the myocardium, ahead of the impulse travelling down the A-V junction over normal pathways. The latter may or may not participate in ventricular activation. If it does, a slender terminal part of the QRS deflection interrupts, or is added to, the delta wave. Unlike the ordinary type of fusion beats which is caused by ventricular interference of two different impulses, one usually originating in the atria and the other in the ventricles, in pre-excitation it is the sinus impulse which splits somewhere on its way toward the ventricles and one (or more) of its offspring takes an anomalous route in reaching them. Pre-excitation results because at least one of the paths which the split sinus impulse uses is a rapid one which avoids physiologic obstacles to impulse conduction through the A-V node. It is for this reason that in pre-excitation the anomalous component invariably forms the initial portion of the resulting fusion beat. On the other hand, like the ordinary ventricular fusion beat, the pre-excitation complex may vary in contour from beat to beat and from time to time, and cause more or less abnormality in the duration and contour of QRS, and in the shape of ST-T. This is clearly brought out in Figure 1. It obviously will also vary from case to case. The similarity of fusion beats caused by ectopic impulses and those caused by pre-excitation may be very close.[1] In fact, the former have been mistaken for the latter even in investigations dealing with the mechanism of pre-excitation.[25]

Apart from the varying degrees of asynchronism of the two fractions of the sinus impulse in the ventricles, other factors may also influence the appearance of pre-excitation beats from case to case, viz., the position of the heart, the alteration of the fundamental electrocardiographic contour by ventricular strain or myocardial infarction, and the location of the point at which the anomalous impulse enters the ventricles. The classic combination of a short P-R and a wide QRS need not always be present to arrive at the diagnosis of a pre-excitation (WPW) syndrome, but then the differentiation from other conditions may not always be possible. Thus records occur with upright P waves, abnormally short P-R intervals, but with a QRS of normal duration which may be interpreted as coronary nodal rhythm[5,31]* as well as pre-excitation beats,[26,27] particularly when attacks of rapid heart action occur in such persons. On the other hand, unquestionable pre-excitation beats may occur with a normal P-R interval[23] or, as is demonstrated clearly in Figure 3, even with an abnormally prolonged one. Persistent ventricular bigeminy, with bizarre beats occurring in every other sinus cycle, just after the sinus P wave, may be caused by ventricular pre-excitation in alternate beats or by late diastolic ventricular ectopic beats. The differentiation of the two is impossible in a single record.[9] In general, the diagnosis of pre-excitation should be made only when respective beats terminate at least two successive cycles, and the P-R of these beats does not change, regardless of its actual length. (Figure 3.) If it does, ventricular fusion beats caused by ectopic impulses, at a rate approaching that of the sinus pacemaker, are more likely.[4,6,8,25,28]

Cardiac Arrhythmias in the Pre-excitation Syndrome. The most frequent disturbance of rhythm, encountered in about three-fourths of all cases,[23] is a supraventricular type of paroxysmal tachycardia with regular spacing and participation of the atria in the rapid ventricular beating. It is, however, not always possible to establish the relationship of the P waves to the QRST preceding or following them and hence to

* Figure 3II of this study.

distinguish between an auricular or nodal type of tachycardia. Next in frequency are auricular fibrillation and flutter, particularly when pre-excitation is present in rheumatic heart disease. Sometimes all three types of auricular arrhythmias can be seen on different occasions in the same patient, as in Figure 2. Ventricular paroxysmal tachycardia appears to be extremely rare. We have observed no case which unequivocally falls into this category. Most of the reported cases are open to an alternative interpretation, the most common being auricular fibrillation with persistent or intermittent ventricular pre-excitation.[17] This problem is dealt with in Figure 1C.

Isolated ectopic premature systoles occur in the pre-excitation syndrome as elsewhere and may be auricular, nodal or ventricular in origin. An unusual instance of ventricular parasystole unrelated to the syndrome is demonstrated in Figure 4. "Passive" ectopic impulse formation (nodal escape and A-V dissociation) can be observed in the pre-excitation syndrome. It is usually elicited by carotid sinus pressure or other types of vagal stimulation applied in order to break a paroxysm of tachycardia or for informative purposes. An example of the latter is shown in Figure 1B. Ectopic nodal beats, premature or escaped, ordinarily show no pre-excitation component[3,11,22,26,29,30] but on occasion they may.[11,24,31]

Finally, rare cases are on record in which ventricular pre-excitation occurred in the presence of second degree A-V block.[12,15,29,32] A unique instance associated with first degree A-V block is reproduced in Figure 3.

The frequency of certain types of cardiac arrhythmias in association with ventricular pre-excitation indicates that they are an integral part of the entire syndrome. Any attempt to explain the syndrome and any concept advanced must account for the occurrence of the phenomenon of pre-excitation under the various circumstances mentioned, and should explain the concomitant disorders of formation and conduction of the cardiac impulse on the basis of physiologically acceptable principles. Careful consideration of the pros and cons of the two main theories as to their applicability to simple and complex arrhythmias leads us to the conclusion that the hypothesis of an accessory A-V connection is most appropriate to account for all of the known aspects of the pre-excitation syndrome. We see, therefore, no present need for implication of any other theory so far advanced.

The Properties of the Anomalous A-V Connection and Their Bearing Upon the Manifestations of the Pre-excitation Syndrome. The accessory atrioventricular bridge appears to be a congenital anomaly since pre-excitation occurs in the newborn and even in the premature,[33] and sometimes in association with other cardiac malformations,[14,19,34] and is possibly a hereditary anomaly since the syndrome has been found in several members of one family.[1,35] It may represent remnants of the embryonic atrioventricular canal wall dislocated during its later incorporation into the adult A-V junction and its differentiation into specific muscle tissue.[5,11] Such anomalous muscle fibres may be remote from the A-V node (eccentric)[1,13,15] or may connect the latter and the common A-V bundle as a short cut to the upper portions of the ventricular septum.[14] Depending on the number, the location and the length of such accessory muscular A-V connections the electrocardiographic pattern of ventricular pre-excitation and the associated manifestations will vary from case to case. Thus an "eccentric" muscle bridge will tend to produce a pronounced delta wave (as seen for instance in Figure 2), or pre-excitation may dominate the ventricular activation so as to minimize the excitation over normal pathways.[36] Conversely, a short anomalous bridge running close to the A-V node and quickly uniting with normal A-V pathways may cause pre-excitation (manifested by a shortened P-R) and yet not alter the mode and duration of ventricular activation significantly.[26,27] An origin of the accessory A-V connection within the A-V node is suggested when ectopic (nodal) beats and beats of sinus origin show an identical pattern of pre-excitation;[21,31] or when pre-excitation is associated with A-V block. (Figure 3.) There may be more than one accessory pathway;[11,18] one may cause pre-excitation, the other may be used preferentially by impulses originating in the A-V node.[37] This could explain the multiplicity of phenomena in our Figure 2.

The property of conductivity in the anomalous A-V bypass may appear early in life and persist over many years[38] or it may be latent at first and become manifest later,[39] spontaneously or when normal conduction pathways are diseased ("acquired WPW syndrome").[1,12,16] Rapid conduction through such fibres may be persistent or intermittent and may be unidirectional or bi-

directional. The fact that in the ectopic anomalous beats of Figure 5 retrograde activation of the atria precedes that of the ventricles can be explained on this basis. Variations in conductivity in the accessory pathway under the influence of nerves and drugs do not always parallel those of the normal pathway.[23] Sometimes only the anomalous path is affected, sometimes only the normal one and sometimes both pathways. Under the last circumstances the effects may be quantitatively different though in the same direction. Parasympathomimetic drugs and digitalis appear to affect adversely only normal A-V conduction, whereas quinidine and pronestyl® depress conductivity through the bypass.[17,29,40,41,42] Such drug effects can be used to verify the presence of pre-excitation in doubtful cases or to unmask other unrelated electrocardiographic alterations.[43] An unusual example of independent changes of impulse transmission in the two pathways achieved by carotid sinus stimulation is shown in Figure 2B.

Independent variations of refractoriness and recovery in the two pathways may influence the pattern of pre-excitation especially during rapid stimulation by auricular premature systoles (Fig. 1B) or in auricular fibrillation. (Fig. 1C.) Such inequality in the conditions for impulse transmission may lead to incomplete penetration of one of the pathways. Retrograde penetration of one pathway by impulses which have passed down the other can occur in this way, and may prevent subsequent stimulation of the former from the atrial end. This mechanism —a variety of concealed conduction—may account for temporary disappearance, or appearance, of pre-excitation during rapid heart action, as exemplified in Figure 1C. Common types of aberrant ventricular conduction are seen especially at the onset of paroxysmal tachycardia (Fig. 1B) and may complicate interpretation of such records.[17]

Since an accessory A-V conduction path may have all these properties it is possible to explain satisfactorily all varieties of rapid heart action associated with pre-excitation. Impulses conducted in a normal fashion through the A-V junction to the ventricles may return back toward the atria via the anomalous path[10] (a variety of re-entry), and continuation of such a re-entry mechanism may initiate and perpetuate rapid heart action. In support of such a view is the fact that in cases in which the onset of supraventricular tachycardia was recorded the last beat preceding the tachycardia was not of the pre-excitation type.[11,44–47] (This is also the case in Figure 1B.) It is our belief that when the path of the circulating wave thus initiated includes a large portion of the atrial myocardium, the atria will take part in the paroxysmal tachycardia. The P wave during this tachycardia may be upright or retrograde. It will be upright when during the atrioventricular reciprocation the circulating wave enters the atria in a region which is remote from the A-V node, and at such a point as to cause atrial activation to take place in a direction similar to normal, that is, from right to left. A retrograde P wave may result either when the circulating wave enters the atria from the left, remotely from both A-V and S-A nodes, under which circumstances the atrial invasion will precede re-entry into the A-V node; or when the anomalous path originates close to or within the A-V node, under which circumstances the atria may not be included in the re-entry path but will be activated in retrograde fashion by impulses radiating from the re-entry sweep. A "nodal" type of paroxysmal tachycardia will then be the result. It is conceivable that in the latter case the atria remain under the control of the sinus node, with A-V dissociation, but this appears to be extremely rare. Under all these circumstances atrial activation takes place in a coordinated manner. However, should the first or one of the later impulses returning to the atria via the anomalous path reach the atria very early in their cycle, while they are partially refractory and in their "vulnerable phase," it may initiate auricular fibrillation[8] (or flutter). It is possible that paroxysmal auricular tachycardias preceding the onset of flutter and fibrillation, as seen in Figure 2, are initiated in this way, and not by way of the re-entry mechanism described.

With the onset of auricular fibrillation (or flutter), A-V reciprocation is stopped and impulses of the fibrillating atria will start to pass down in rapid irregular sequence either over the normal or the anomalous path, depending on their state of recovery from preceding stimulation. Since each impulse passing down one path has a tendency to penetrate in retrograde fashion into the other, thus rendering it refractory,[17] normal or anomalous, activation of the ventricles will continue for some time until, during a longer ventricular pause, the other pathway recovers and becomes dominant. (Fig. 1C.) Due to this mechanism, the anomalous

beats during auricular fibrillation are usually wider than during sinus rhythm, being entirely composed of the delta wave—the ordinary fusion beats of pre-excitation occur only at the time of transition of one type of ventricular excitation into the other.[17]

The occurrence of pre-excitation beats during auricular fibrillation, in which there is no synergic auricular contraction, is a strong argument against the concept that in sinus rhythm with pre-excitation auricular contraction by mechanical stimulation causes premature discharge of an irritable ventricular focus and that the impulse so generated interferes with the sinus impulse travelling only over normal A-V paths. It favors the view that an anomalous path must be involved. Another strong point favoring the concept of anomalous A-V conduction, and opposed to the concept of an irritable ventricular focus, is the occurrence of pre-excitation complexes in cases with A-V block. In instances of second degree A-V block[12,15,29,32] with dropped ventricular beats it is hard to conceive how failure of response of the supposed ventricular focus occurs simultaneously with failure of the sinus impulse to pass the A-V junction. In the instance of first degree A-V block illustrated in Figure 3 latency of response of the ectopic focus synchronized with the delay of A-V conduction would have to be postulated, a highly improbable circumstance. Implication of an A-V conduction disturbance affecting two conduction pathways, one of which is anomalous, offers a much more reasonable and simple explanation. It is possible that the impulse might be delayed or stopped simultaneously in both pathways. (Fig. 1B.) When, however, the anomalous path is assumed to originate in or below the A-V node, only one area of block, within the node, need be postulated.

On rare occasions ectopic beats have been observed in this syndrome which partly or entirely match the contour of the sinus beats with pre-excitation but occur unrelated to a P wave,[9,29] are linked at a shortened P-R to an anomalous P[9,48] or are preceded by a frankly retrograde P at an abnormally short P-R.[49,50] An example of the latter is shown in Figure 5. Such beats, it would appear to us, originate in the anomalous path itself.

The recognition that impulse formation may on rare occasions take place in the anomalous fibres does not contradict the concept that the principal role of the accessory path is to accelerate A-V conduction and thus to produce the syndrome of pre-excitation. As in ordinary A-V junctional tissue, two properties may exist in a congenitally displaced portion of it. It appears that the property of conductivity of such fibres, both in a forward and in a retrograde direction, is the primary factor responsible for the usual spontaneous manifestations of the syndrome, and that the property of rhythmicity ordinarily is absent or dormant. Rarely, the latter too may come into operation, and then causes isolated premature systoles, or takes over the pacemaker function of the heart, as occurred in the case illustrated in Figure 5.

It has been stated that "delayed anomalous conduction which would furnish the final link in the chain of evidence for a functioning structural accessory (A-V) pathway has never been observed;'[22] and that "there is no proof that stimuli originate in the anomalous pathway."[12] We believe that these two missing links are provided in the two observations reproduced in Figures 3 and 5. Future similar demonstrations of such a dual function of the supposed anomalous fibres should prove the case in favor of the presence of an accessory A-V connection in the pre-excitation syndrome.

SUMMARY AND CONCLUSIONS

1. Five selected cases of the pre-excitation (WPW) syndrome which exhibited various types of disturbance of formation and conduction of the cardiac impulse were submitted to detailed analysis and examined as to the applicability of the two fundamental concepts of pre-excitation, the hypothesis of an hyperexcitable ventricular focus and that of an anomalous A-V conduction bypass.

2. In all five instances the hypothesis of one or more anomalous A-V connections proved most appropriate to account for all aspects of the pre-excitation syndrome, including various simple and complex arrhythmias. This was true even in the presence of a ventricular parasystolic center.

3. In one case in which ventricular pre-excitation occurred in association with an abnormally prolonged P-R interval, the accessory A-V junction was considered to originate in the lower portion of, or below, the A-V node. In another instance, intermittent spontaneous impulse formation in an accessory A-V bridge had to be postulated to account for all characteristics of the ectopic beats occurring as

sporadic premature systoles or in the form of a paroxysmal tachycardia. Delayed anomalous excitation, and impulse formation in the anomalous bypass, demonstrable in these two cases, represent two missing links postulated as additional evidence for the concept of an accessory A-V connection.

4. On this basis it would appear that the anomalous muscular A-V connection present in patients with the pre-excitation syndrome has the special properties ordinarily attributed to specific cardiac tissue, viz., conductivity and rhythmicity. The property of conductivity predominates and is the one which is responsible for the common spontaneous manifestations of ventricular pre-excitation, including the various associated disorders of rhythm. The property of rhythmicity, when present, ordinarily is dormant. In exceptional instances it may become manifest and lead to isolated ectopic beats or to ectopic rhythms.

Acknowledgment: We are indebted to Dr. S. Contro for permission to include his observation (Case 3), and to Dr. R. Langendorf for his comments.

ADDENDUM

Since this study was submitted for publication further proof of the existence of accessory A-V connections was provided by Lev, Gibson and Miller (*Am. Heart J.*, 49: 725, 1955) by careful histologic study of the entire conduction system and both A-V rings in a case of Ebstein's disease associated with ventricular pre-excitation. About the same time, Borduas, Rakita, Kennamer and Prinzmetal (*Circulation*, 11: 69, 1955) advanced the view that the Wolff-Parkinson-White syndrome is an example of "accelerated A-V conduction," a hitherto unrecognized abnormal mechanism operating within the A-V node. As evidence for this concept they presented short strips of clinical and experimental electrocardiograms which, in our judgment, may be interpreted as: (1) nodal escape and nodal rhythm leading to intermittent A-V dissociation, (2) ventricular premature systoles interfering with A-V conduction of sinus impulses, or (3) 2:1 A-V block in which the P-R duration exceeds the P-P interval.

ILLUSTRATIVE APPENDIX

Figure 1. All strips are lead I recorded on different occasions in a sixty year old man with hypertension.

In strip A, during sinus rhythm (rate 64), the pre-excitation syndrome is revealed by the combination of a short P-R (0.08 sec.) and a small delta wave widening QRS to 0.10 sec. Four such sinus beats are seen in strip B viz., the first two and the fifth and seventh beats (S-T is depressed, unlike those in A). Each of these beats except the first is followed by two premature P waves (pairs of auricular premature systoles) giving rise to an upright notch in the ST-T of the preceding beat. The first of these pairs of premature P waves is conducted and leads to a pre-excitation beat with an exaggerated delta wave and a negative ST-T. In these beats a greater amount of ventricular muscle is stimulated in an anomalous fashion than in the sinus beats. The second P wave of the pair occurs at a shorter P-P interval than the first. In the second group it is non-conducted; in the first and third groups it is conducted, giving rise to a totally different diphasic ventricular complex, with a tall upright T wave and a QRS of 0.10 sec. but without a delta wave. In these beats ventricular activation takes place entirely over ordinary A-V conduction pathways but this is altered by aberrant ventricular conduction such as occurs commonly in early auricular premature systoles. In the third group, this beat, in turn, is followed by a run of regularly spaced beats (rate of 188) of normal QRS duration (0.07 sec.) with a P wave preceding each ventricular complex (auricular paroxysmal tachycardia).

The variety of ventricular complexes encountered in strip B and their recurrent grouping can be explained appropriately by assuming the presence of two A-V conduction pathways having independent characteristics of refractoriness and recovery. When the rate is slow (as in A), conduction over the ordinary pathways dominates over that through the anomalous one, resulting in the appearance of only a small delta wave. This dominance of the ordinary path lessens in the first of the premature beats (in B), apparently because recovery is faster in the bypass. However, in the second of the pair of conducted premature beats the dominance is reversed in favor of normal A-V conduction. This paradoxic behavior of the two pathways cannot be accounted for by simple recovery phenomena. It may be that the cycle length preceding the beat ahead of a premature impulse exerts a more pronounced influence upon the duration of the refractory period of the

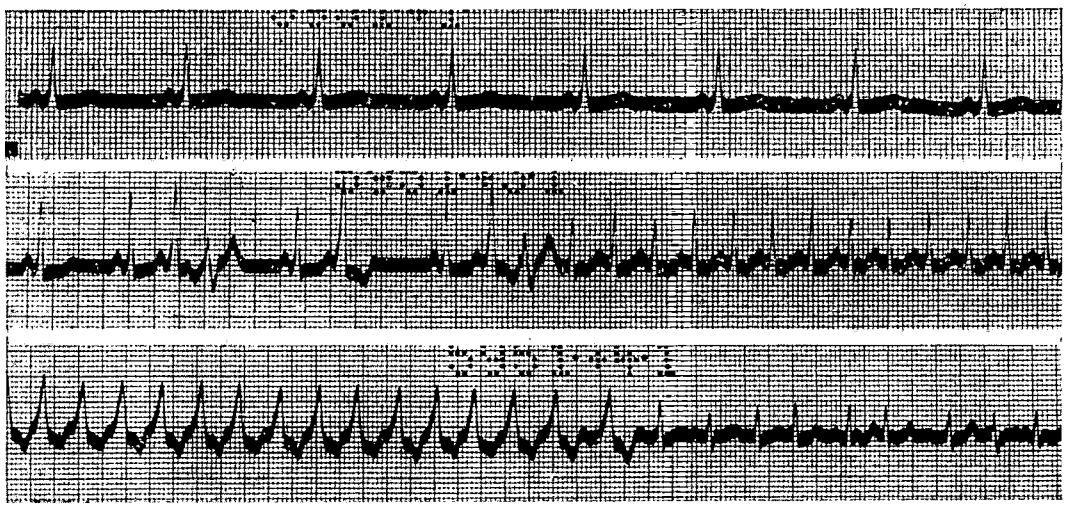


FIG. 1. A case of pre-excitation (WPW) syndrome (A) associated with auricular premature beats and a paroxysm of supraventricular tachycardia (B) and a paroxysm of auricular fibrillation (C). (See text.)

ordinary path than of the anomalous one, so that after two successive short cycles conduction is enhanced over the normal A-V conduction pathways.

The second impulse of the pairs of premature systoles occurs so early that it leads to the usual type of aberrant ventricular conduction and, on one occasion, does not yield a ventricular response. In the latter case it must have been stopped in both pathways.

The absence of the delta wave during the stabilized period of rapid heart action at the end of strip B shows that only normal A-V conduction pathways are used in transmitting the impulse from the atria to the ventricles. Apparently the second premature impulse in the last group of premature beats, after activating the ventricles, succeeded in penetrating the bypass backward to reach and reactivate the atria, and thus initiated continued atrioventricular reciprocation. Obviously because the bypass was used for retrograde conduction it could not be entered by the rapid auricular impulses.

In strip C the diagnosis of auricular fibrillation is made on the basis of the irregular rapid ventricular beats and the presence of irregular undulations (f waves) clearly seen in the second half of the strip. The run of bizarre unevenly spaced ventricular complexes in the first half of the record is not due to ventricular paroxysmal tachycardia (which it resembles) but to conduction of auricular impulses through the anomalous bypass. This is established by comparing these beats with the pre-excitation beats in A and B. At first, during auricular fibrillation, the delta wave occupies the entire upstroke and QRS measures 0.14 second, suggesting that the ventricular activation is effected entirely by the anomalous path. Apparently, impulses constantly are penetrating into the normal A-V conduction pathways from the ventricular end and render the A-V node refractory to impulses from the fibrillating atria (a variety of concealed conduction). However, in the middle of the record, when the ventricular rate transiently slows, one of the auricular impulses succeeds in completely traversing the normal A-V pathways in a forward direction and immediately conditions are reversed. This impulse after reaching the ventricles then penetrates into the bypass in retrograde fashion and so renders this path refractory to auricular impulses. This continues and the result is persistent normal forward A-V conduction up to the end of the record. The slight variations in the QRST contour during this latter period is a common occurrence in ordinary auricular fibrillation.

In summary, this case illustrates: (1) the variety of types of ectopic auricular impulse formation which may accompany the pre-excitation syndrome; (2) the variations in QRST contour caused by the availability of an accessory conduction pathway; and (3) the problem of differentiating ventricular paroxysmal tachycardia from the combination of pre-excitation with auricular fibrillation.

The fact that ventricular pre-excitation occurs during bouts of auricular fibrillation excludes



FIG. 2A and B. Ectopic supraventricular rhythms (A) and effects of carotid sinus pressure (B) in the pre-excitation (WPW) syndrome. (See text.)

the view that the anomalous component of QRS is due to mechanical stimulation by the atrial contraction of an irritable focus in the ventricles, since such a synergic contraction of the atria is not present in auricular fibrillation. On the other hand, it is no more difficult to explain the occurrence of auricular fibrillation (and flutter) in the pre-excitation syndrome by the assumption of an accessory A-V pathway than that of other forms of paroxysmal supraventricular tachycardia. If a single auricular impulse, having traversed the normal A-V conduction pathway, returns to the atria via the anomalous bypass and reaches the atria early, in the "vulnerable period," auricular fibrillation (or flutter) may be initiated instead of a reciprocating mechanism between atria and ventricles which causes regular supraventricular paroxysmal tachycardia.

Thus the assumption of an anomalous A-V bypass can best account for all varieties of disturbance of rhythm and alterations in QRST contour encountered in this case.

Figure 2. The records shown (all in lead II, with time lines indicating 0.05 sec.) are portions of numerous tracings obtained in a twenty-year old man with rheumatic heart disease.

The variety of A-V conduction patterns ob-

served during periods of different rhythm are shown in A. In the top strip there is sinus bradycardia (rate 50) with normal A-V (P-R, 0.20 sec.) and intraventricular (QRS, 0.08 sec.) conduction. In the second strip in which the sinus rate varies between 57 and 67, the last three beats reveal a contour characteristic for ventricular pre-excitation in that QRS is prolonged to 0.14 sec. by a distinct delta wave. The latter starts immediately after a small terminal notch of the P wave so that P-R is shortened to 0.12 sec. The demarcation of the anomalous from the normal component of ventricular activation is very sharp. The first two beats of this strip differ in contour from both the normal (in the top strip) and the pre-excitation beats. Their QRS is 0.11 sec. and their P-R distance varies. These are ectopic (nodal) beats, activating the ventricles during a period of A-V dissociation. The reasons for the slight aberration of their contour is discussed later. The lower four strips exemplify various types of disturbances of atrial rhythm observed in this patient. In the sequence from above down, there is, first, a paroxysmal auricular tachycardia (rate 172) with 1:1 A-V conduction. In the next strip the same tachycardia is seen at a slightly faster rate (180) with irregular A-V conduction varying between 3:1 and 5:1 ratios. In the next strip there is auricular flutter (rate 300) with regular 4:1 A-V conduction, and in the last strip auricular fibrillation has developed (rate about 400) with a slow irregular ventricular response (average 60). All these varieties of rapid auricular action cause ventricular activation via normal A-V conduction pathways as evidenced by the normal contour and duration (0.08 sec.) of QRS; (compare with top strip).

Various effects of carotid sinus pressure upon A-V conduction and ventricular activation during sinus rhythm are shown in B. In the top strip, which starts with a pre-excitation beat, carotid sinus stimulation (↓) causes slowing of the sinus rate. The node escapes and causes, by A-V interference, a period of A-V dissociation until, after release of carotid sinus pressure (↑), the ventricular pre-excitation by the sinus impulse is resumed. The nodal beats show the same slight aberrant contour seen at the beginning of the second strip of A. In the lower strip, the effect of carotid sinus pressure during the pre-excitation is more marked and causes a period of complete standstill of 3.2-second duration because both sinus and A-V nodes are suppressed. The first beat after the onset of the carotid sinus pressure (↓), and the three beats following the pause, differ in contour from the pre-excitation complexes ordinarily seen in this patient in that the amplitude of the terminal narrow portion is smaller, whereas the delta wave is somewhat taller.

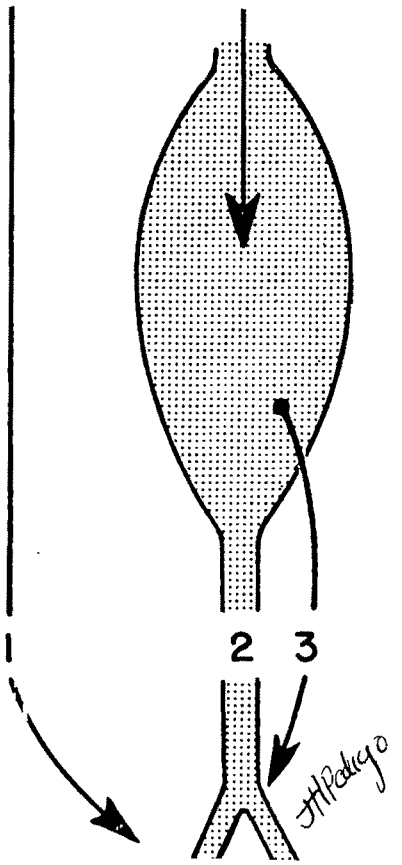


FIG. 2C. A diagrammatic representation of the three A-V conduction pathways assumed to be present in Figures 2A and 2B.

The observations in this patient's records can be summarized as follows: Four types of ventricular beats are present, viz., (1) the normal type seen with both sinus and ectopic rapid auricular rhythms; (2) ordinary pre-excitation beats seen intermittently only during sinus rhythm; (3) modified pre-excitation beats occurring with carotid sinus stimulation, and (4) ectopic supraventricular beats, nodal in origin, with aberrant ventricular contour. Carotid sinus stimulation, besides altering the contour of the pre-excitation beats, caused sinus slowing with nodal escape, or simultaneous standstill of both the sinus and A-V nodes.

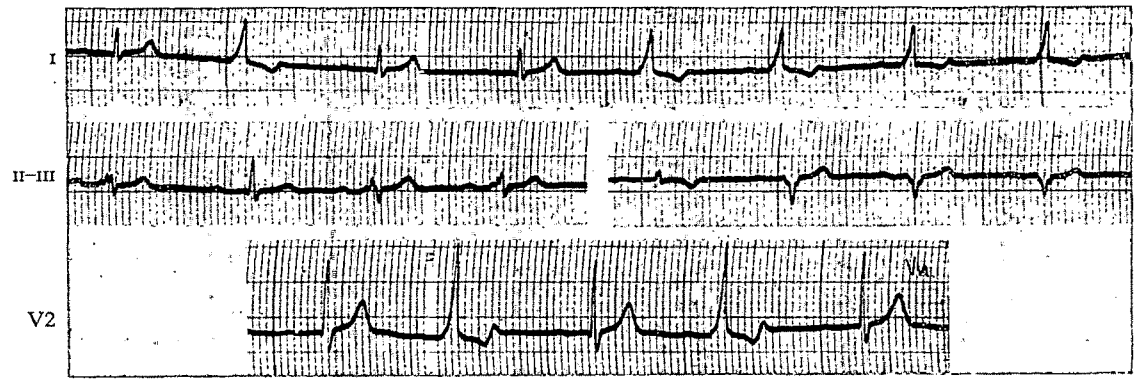


FIG. 3. Pre-excitation (WPW) syndrome with prolonged P-R interval in a thirty-two year old man, asymptomatic and without clinical evidence of heart disease. (Time lines 0.05 sec.) (See text.)

While it seems impossible to account for this combination of phenomena on a uniform basis by implicating an hyperexcitable ventricular focus, they can be explained on the assumption that there are three A-V conduction pathways, as indicated in the accompanying diagram 2C. In this diagram (1) represents an anomalous pathway bypassing the A-V node, (2) represents the ordinary normal conduction pathway, and (3) represents a preferential path near (2) used, as is often the case, by impulses of nodal origin. The sinus impulses, in stimulating the ventricles, use either path (2)—with a normal QRS resulting; or both (1) and (2)—with a pre-excitation beat resulting. Nodal impulses stimulate the ventricles over their preferential pathway (3) whereas the ectopic auricular impulses (during auricular tachycardia, flutter and fibrillation) can use pathway (2) only. Pathway (1) is refractory during the rapid auricular activity because it is persistently penetrated in a backward direction from its ventricular end by the rapid auricular impulses returning to the atria, a re-entry mechanism which may be responsible for initiation as well as perpetuation of the rapid atrial activity. At times the A-V node escapes because the sinus rate slows below the inherent rate of the node. As a consequence, the forward impulse transmitted from the node over pathway (3) dominates the ventricles. The retrograde nodal impulse interferes with the oncoming sinus impulse and causes A-V dissociation. The absence in nodal beats of a delta wave (indicating ventricular stimulation by an impulse traversing pathway (1)) means that the nodal and sinus impulse interfere with each other either above, within or at the ventricular end of pathway (1). Either of the two impulses (the forward or retrograde one) may penetrate the anomalous pathway, without traversing it, and this would represent a variety of concealed A-V conduction.

The effect of carotid sinus stimulation upon the contour of the pre-excitation beats is the result of depression of the conductivity of pathway (2), so that the sinus impulse which divides to use both pathway (1) and pathway (2) travels more slowly over the latter and, as a consequence, more of the ventricles will be dominated by the impulse reaching it over pathway (1). Therefore, the resulting ventricular fusion beats will show a greater delta and a smaller normal part in the QRS complex.

Again, the hypothesis of anomalous A-V bridges appears to be the only one which can satisfactorily explain all the facts observed, i.e., the variable contour of ventricular beats under different conditions as well as the multiplicity of abnormal rhythms encountered.

Figure 3. In each lead two types of ventricular complexes are seen, one of which is normal whereas the other has a contour typical for ventricular pre-excitation with QRS prolonged to 0.12 sec. The P-R interval of both is abnormally prolonged, although to a lesser degree (0.24 sec.) in the pre-excitation beats than in the beats with normal ventricular activation (0.30 sec.). The interval from the onset of P to the end of QRS is the same in both (0.36 sec.). While in V2 the pre-excitation syndrome could be questioned, and late diastolic ectopic ventricular beats with A-V interference might be suspected, the presence of pre-excitation is established in the three standard limb leads in which anomalous beats at a constant P-R occur several times in succession.

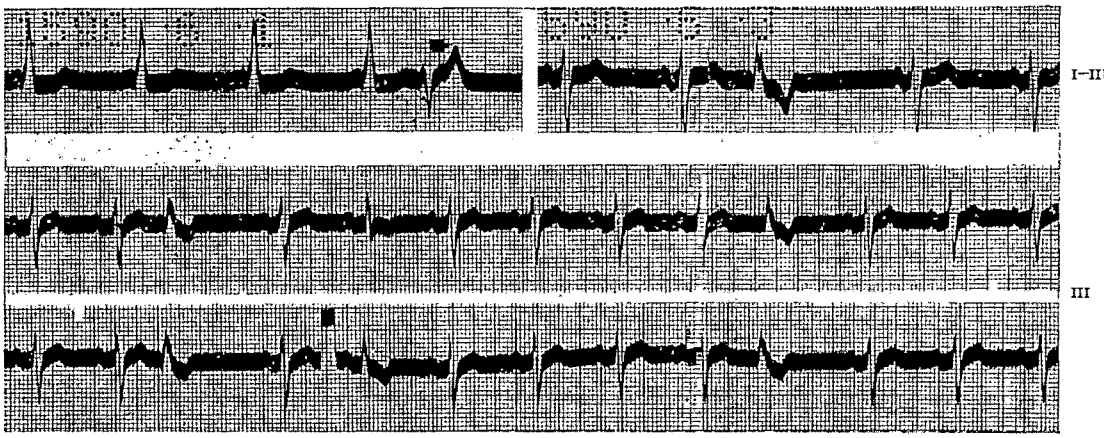


FIG. 4. Ventricular parasystole in the pre-excitation (WPW) syndrome in a seventy-two year old man with chronic coronary insufficiency.

This case is unique because it illustrates that ventricular pre-excitation may take place with a P-R interval still abnormally long. Thus an abnormally short P-R is not a necessary part of the syndrome. Moreover, this observation necessarily leads to certain conclusions, in this particular case and in general, as to the mechanism of ventricular pre-excitation. With such a long P-R interval of the pre-excitation beats it is very difficult to conceive any type of stimulation —mechanical or electrical—of an irritable ventricular focus by atrial activity occurring ahead of the impulse traveling down over normal A-V conduction pathways. Thus one of the two principal concepts advanced seems to be ruled out. If, on the other hand, an anomalous connection is present between atria and ventricles, the block causing the prolonged P-R in both normal and pre-excitation beats must be located above its origin unless simultaneous depression of about equal degree is assumed in both the normal and anomalous pathways; this last is extremely unlikely. Since abnormal impedance to A-V conduction is most commonly located in A-V junctional tissue, and particularly in the A-V node, it can be concluded that the anomalous path, over which the impulse bypasses part of the obstacle of the A-V junction, arises either in the lower portion of the A-V node or below it.

This case may therefore be considered to be a strong link in the chain of evidence in support of the concept ascribing ventricular pre-excitation to an anomalous A-V bridge bypassing the obstacle to impulse conduction in the A-V node, entirely or in part.

Figure 4. The two leads shown in the upper row have typical earmarks of ventricular pre-excitation: upright P waves (rate 59) linked by a constant abnormally short (0.10 sec.) P-R interval to abnormal QRS complexes widened to 0.12 sec. by a delta wave. The last beat in lead I and the third beat in lead III occur prematurely and have a contour totally different from the dominant pre-excitation beats. Their coupling to sinus beats differs and so does their relation to P. In lead III a sinus P wave, in lead I a premature P, can be identified within their S-T. On this basis the diagnosis of ventricular premature systoles is made, causing A-V interference (in III), or retrograde conduction to the atria (in I), depending on the length of their coupling.

Because of the variability of the coupling of the ectopic beats a long record of lead III was obtained on the next day, two continuous portions of which are shown in the two lower strips. (The last beat of the middle strip is reproduced as the first beat of the lowest strip.) The dominant sinus beats (rate 79) and most of the ectopic beats resemble those seen previously in lead III. However, two beats (the fifth in each strip), which follow a sinus P wave, have a contour intermediate between the two types. Both start with a delta wave but in the former the terminal portion of QRS resembles the sinus beats while in the latter it resembles the ectopic beats. These are ventricular fusion beats with ventricular interference between a sinus impulse and an ectopic impulse occurring subsequent to the ventricular pre-excitation. When the intervals between ectopic beats, and ectopic

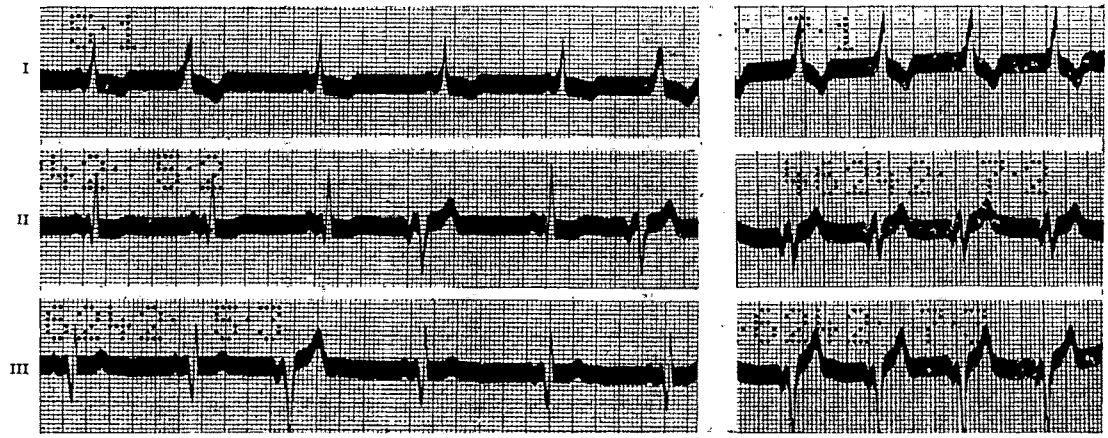


FIG. 5. Pre-excitation (WPW) syndrome with ectopic beats apparently arising in the anomalous path, in a sixty-seven year old man with chronic bronchopulmonary disease. (See text.)

and fusion beats, were measured in the entire record the presence of a ventricular parasystolic focus, discharging at a regular rate of 31, could be established.

An irritable ventricular focus responding with premature impulse formation to the atrial contraction has been invoked to account for the phenomenon of ventricular pre-excitation. Since in this case the autonomous "protected" rhythmic character of the ectopic ventricular focus was unquestionably demonstrated, it cannot be involved in producing the ventricular pre-excitation of the sinus beats. The protection from other impulses which permits maintenance of its steady rhythm renders such an implication impossible. Thus premature ventricular systoles can occur coincidentally with the pre-excitation syndrome, as in any other condition.

Figure 5. In A, in the dominant sinus rhythm (rate 60), the presence of the pre-excitation syndrome is diagnosed on the basis of a short P-R (0.10 sec.) and the presence of a delta wave. The anomalous component, widening QRS to 0.12 sec., starts as an upright deflection in all three leads, largest in I. In addition, there are premature systoles in each lead preceded by premature P waves of retrograde contour, with a P-R less than that of the sinus beats. These beats start with a deflection similar to the delta wave of the sinus beats but unlike the latter, its course is not interrupted by the slender normal positive component of simultaneous ventricular invasion via normal A-V pathways. Thus the entire downward deflection of QRS of the premature beats in leads II and III seems to be caused by the impulse entering the ventricles anomalously.

In B, obtained in the same patient on a different occasion, all beats resemble the isolated premature beats of A in regard to P, P-R and QRST. They recur at a regular rate of 75, which is faster than the previous sinus rate. Hence, it appears that the same ectopic focus which produced the sporadic premature beats in record A has taken over the control of the entire heart in record B since its impulses occur in regular sequence and at a rate faster than the primary sinus pacemaker.

The main problem in this case is in identifying the location of the ectopic focus and determining its bearing upon the possible mechanisms of the pre-excitation syndrome. The two relevant facts in this connection are: (1) that the ventricular complex of the ectopic beats starts like the sinus beats but the final element of the sinus beats is missing; (2) that there is a retrograde premature P wave ahead of these beats with a P-R shorter than that of the sinus beats. Since the criterion of use of the normal A-V path (the slender upright terminal part of sinus QRS) is absent, it is concluded that the ordinary A-V conduction path is not being used in the ectopic beats. If this is so, then these beats cannot be nodal in origin (even though they have some of the earmarks of nodal origin); otherwise, some element of normal A-V conduction should be visible in the QRS of these beats since an impulse arising in the A-V node has to traverse it. It is thus the absence of signs of ventricular fusion in

the ectopic beats which excludes their origin in a nodal focus in this instance. The ectopic focus must therefore be localized to an area which permits: (1) rapid retrograde stimulation of the atria; and (2) stimulation of the ventricles over anomalous pathways, either completely when the ectopic focus dominates the heart, or partially when the sinus rhythm is effective. The logical conclusion is that the ectopic focus is located within an anomalous A-V bridge.

This record is significant in regard to the concept of the pre-excitation syndrome. It shows that the anomalous path not only permits impulses to bypass the ordinary conduction pathway but also can initiate impulses itself or, more properly, contains tissues which have this potential property. Ordinarily, the property of conductivity seems to be dominant in the anomalous bypass and that of impulse formation is latent or absent. If our interpretation is correct, the A-V bypass in this case showed both conductivity and spontaneous rhythmicity. The former led to ventricular pre-excitation during sinus rhythm, the latter to sporadic premature beats and a paroxysm of ectopic heart action in which retrograde conduction was as rapid as forward conduction. The demonstration of such a dual function provides further support for the assumption of an accessory A-V connection in the pre-excitation syndrome.

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Gross Hematuria in Sickle Cell Trait and Sickle Cell Hemoglobin-C Disease*

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HEMATURIA in sickle cell anemia has been ascribed to congestion of small vessels, thrombosis, stasis and infarction necrosis in the kidneys.[1] These changes have been linked to the abnormal sickle shape of the red cells in this disease. Pathologic evidence has been obtained in these cases of puckering of the renal surface from obliteration of engorged arteries, destruction of glomeruli from blockage of glomerular arteries, and scarring in the apices of the pyramids as a result of capillary bed blockage by sickled red cells between collecting tubules. Kimmelstiel[2] described the changes as temporary capillary stasis after shock, and spasm with ischemia and necrosis as a result of plugging of capillaries by sickled cells, resulting in vessel wall injury and, rarely, in true capillary thrombi.

The incidence of hematuria with sickle cell anemia has been reported by Henderson[3] as three in fifty-four cases and by Patterson, Wilson and Diggs[4] as two in 142 cases.

Abel and Brown[5] in 1948 reported a case of gross hematuria with a red cell count of 4,000,000, back pain and persistent filling defect in the left kidney in which eventual nephrectomy was performed. This could be considered a doubtful case of hematuria in sickle cell trait. Goodwin[6] reported four cases of hematuria in sickle cell disease, several of which could have been due to sickle cell trait. It is also possible that some of his cases may have been sickle cell hemoglobin-C disease. He also reviewed the hospital records from 1938 to 1947 and found nineteen cases of essential hematuria in Negroes, six of whom had sickle cell trait, two were negative and the rest were not tested. This author postulated minute thrombosis leading to stasis and vascular leakage as the underlying pathology. Greecy et al.[7] reported a case of sickle cell trait with hematuria, in which nephrectomy was performed. The pathologist reported a grossly normal kidney with clumping and sickling of red cells in the papillae on microscopic section. Pyelography and cultures for acid-fast organisms were negative. Ryan and Fuller[8] reported the case of a thirty-one year old Negro with sickle cell trait who had painless hematuria and in whom cystoscopy and retrograde studies were negative. Nephrectomy was performed, examination of the specimen showing small hemorrhagic areas in the mucosa of the calyces. Harrison and Harrison[9] reported on nine patients with unilateral hematuria and sickle cell disease. Most of the cases represented sickle cell trait; one had sickle cell anemia. Nephrectomy was performed in four cases and pathologic examination revealed petechiae, edematous papillae, hemorrhages, vascular dilatation and sickling in the vessels. In one case increase in size and proliferation of tubular epithelial cells was seen. The authors favored Kimmelstiel's shock theory of peripheral vascular spasm, leading to ischemic necrosis of tissue by "packing" of capillaries with sickled cells causing vessel wall injury and ultimate infarction. They also suggested an additional factor of inherent kidney defect, vascular or otherwise, in the presence of sicklemia as a cause of these changes. Lund et al.[10] reported seven cases of gross hematuria; six were unilateral and one was eventually bilateral. Two nephrectomies were performed and pathologic examination revealed sickle cells in the engorged small vessels, venules, capillaries, glomeruli and areas of hemorrhage. Their studies included acid-fast cultures, Papannicolaou and hemoglobin alkali resistance

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determinations. They found that bleeding was more common on the left side.

Recent remarkable advances in the electrophoretic study of the hemoglobinopathies have clarified many aspects of sickle cell disease.[21–23] Many of the unusual cases of sickle cell crisis without anemia, with transient anemia or with persistent splenomegaly can now be explained by the presence of sickle cell hemoglobin-C disease. In 1929 Levy[11] reported cases of sickle cell disease, one of which could easily fit this category. (Table I.) Bauer[12,13] stressed the importance of circulatory stasis from engorgement of small vessels by sickled red cells as cause of symptoms, and minimized hemolysis. Review of his case reports suggest that one of these could have been sickle cell hemoglobin-C disease. Green and Lockhard[14] reported symptomatic sickle cell disease without persistent anemia, one case with a very unusual hemoglobin pattern electrophoretically; these three cases could well have been sickle cell hemoglobin-C disease. Smith and Lockhard[15] reported the results of filter paper electrophoresis of hemoglobin in two cases of hematuria associated with sickle cell hemoglobin-C disease. Diggs and Jones[16] reported the case of a forty-eight year old Negress in whom abdominal pain, joint pains, fever, icterus, petechiae and coma developed after an alcoholic debauch, with sickling in the peripheral blood smear, targeting of the red cells and splenomegaly. It was postulated that the sickling process was precipitated by the alcoholic excess.

TABLE I

CASES IN LITERATURE WHICH MAY HAVE BEEN SICKLE CELL HEMOGLOBIN-C DISEASE

Authors	Age of Patient (yr.)	Hemoglobin	Red Blood Cell Count	Spleen	Icterus	Crises	Targeting, Sickling	Electrophoresis
Levy (1929)	8	80%	5.0	3 cm.	+	+	−−	.....
Bauer (1943)	24	78%	5.15	550 gm.	?	+	.....	.....
	26	?	?	620 gm.	..	++	.....	.....
Green and Lockhard (1951)	50	12 gm.	5.47	0	+	+	−−	Abnormal, not SS or SA
	22	11 gm.	5.64	Tip palpable	+	+	−−	.....
	24	12.3 gm	5.05	++	+	+	−−	.....
Diggs and Jones (1952)	48	11 gm.	3.12	400 gm.	+	Severe crisis after alcoholic bout	+++	.....
Smith and Lockhard (1953)	35	.....	3.8	+	−	Hematuria	.....	SC − FH 3.8%
	16	.....	....	.....	..	Hematuria, knee pains	.....	SC − FH 6%

A summary of these possible instances of sickle cell hemoglobin-C disease, as reported in the literature, is given in Table I. Five additional cases of sickle cell trait and three of sickle cell hemoglobin-C disease manifesting gross hematuria are described in this communication. Filter paper electrophoresis and fetal hemoglobin studies were performed. (Fig. 1.) Urologic studies and results of nephrectomy in several of these cases are being reported separately in more detail.[18]

CASE REPORTS

CASE I. C. A., a thirty-eight year old man, was admitted to the Hines V. A. Hospital in June, 1953. He stated that he had had a bout of gross hematuria in September, 1950, treated at

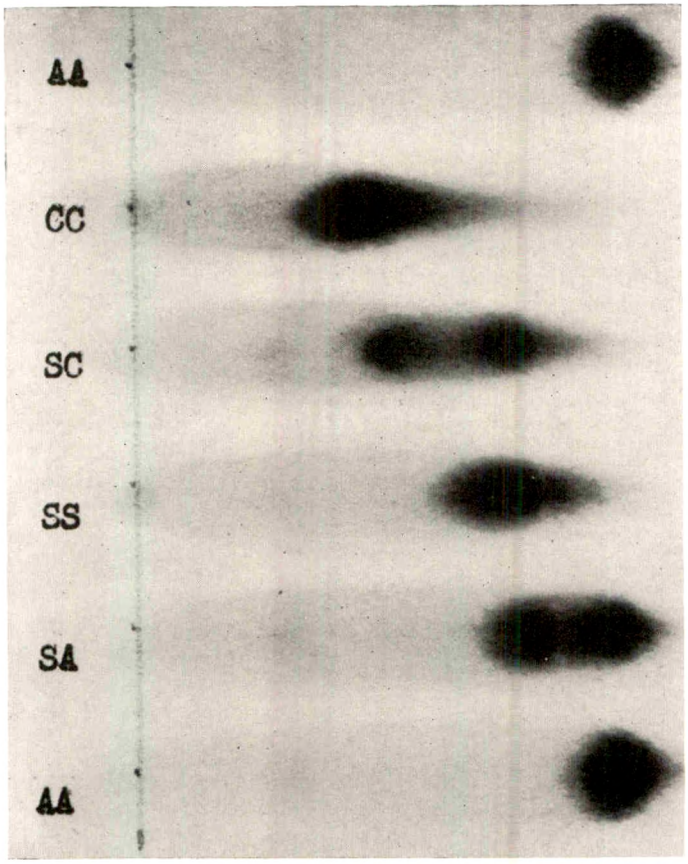
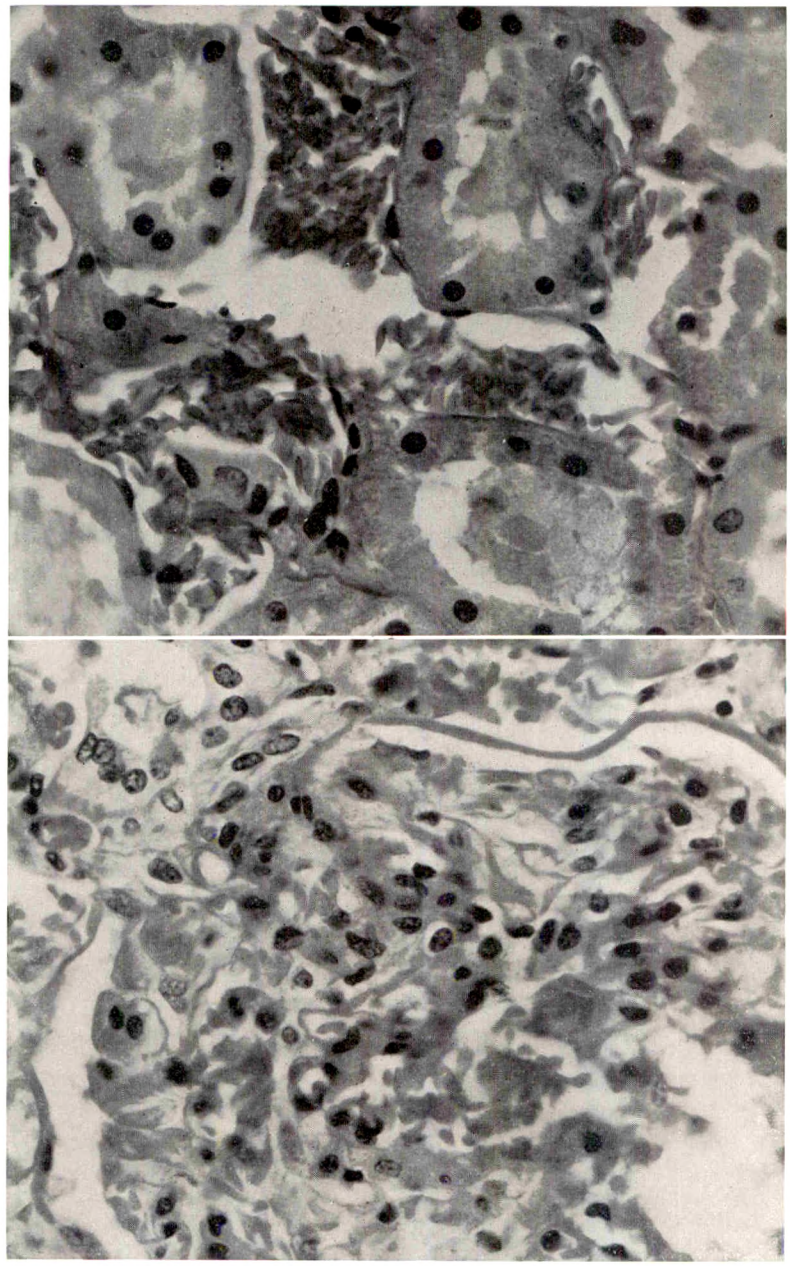


FIG. 1. Photograph of filter paper strip showing types SA and SC hemoglobins as seen in our patients. Types SS, AA, and CC hemoglobins are shown for comparative purposes.

the V. A. Hospital in Memphis, Tennessee. There were no further episodes of hematuria until June, 1953, when he noted dark urine for one week followed by grossly red urine one week preceding admission to the hospital. Physical examination was not remarkable. Hepatosplenomegaly was not present. The sickle cell test was positive; fetal hemoglobin was normal; filter paper electrophoresis revealed type AS hemoglobin. Evidence of hemolysis was lacking; blood chemical studies and genitourinary examinations were normal. Since his discharge from the hospital in June, 1953, the patient has had two episodes of hematuria to date. His last red blood cell count was 4,700,000, with 14 gm. of hemoglobin. Subsequent urologic examinations have been negative.

CASE II. J. G. B., a thirty year old Negro man, was first hospitalized at the Hines V. A. Hospital in 1949, on the Genitourinary Service. He gave a history of recurring episodes of bloody urine since 1944. Because of abnormal intravenous and retrograde pyelograms, exploration of the left kidney was performed but no abnormalities were found.

In June, 1951, he was seen on follow-up examination and a sickle cell preparation was found to be positive. He has had frequent episodes of hematuria since his original admission in 1949. In all instances the hematuria has been painless and has cleared spontaneously. Physical examination during a recent hospitalization revealed a well developed, well nourished, Negro man with no abnormal physical findings. Laboratory studies showed a red blood count of 3,800,000, with 12.5 gm. of hemoglobin. The urine has varied between grossly bloody and negative. Chest x-rays have been essentially normal. Filter paper electrophoresis revealed type AS hemoglobin. Alkali denaturation technic for fetal hemoglobin was normal. Subsequent intravenous pyelograms have been normal and



2A

2B

FIG. 2. A and B. Photomicrographs of sickle cells. A, in renal tubules; B, in glomerulus of kidney in one case of gross hematuria.

urine culture for acid-fast bacillus have been negative.

CASE III. J. L., a twenty-four year old Negro man, first noted gross hematuria in November, 1948, when he was serving in the Army as a laboratory technician. An intravenous pyelogram taken at this time was negative. In March, 1949, complete genitourinary studies, including urine cultures, intravenous pyelograms and retrograde pyelograms, were carried out and a defect was found at the left ureteral pelvic junction suggestive of extrinsic pressure. Cystoscopic findings revealed fresh blood coming from the right ureter. The patient continued to have gross and constant hematuria. In August, 1949, right nephrectomy was performed. The patient

thereafter noted no hematuria and was well until August, 1952, when he was hospitalized at the Hines V. A. Hospital for gross painless hematuria. Throughout his hospital stay he showed alternating gross and microscopic hematuria; blood transfusion was necessary because of a low red cell count at one time during his hospital stay. Physical examination revealed a well developed, well nourished, Negro man who did not appear acutely ill. The heart and lungs were normal. There was no enlargement of the liver or spleen. Laboratory findings revealed a normal hemogram. The platelet count was 182,000. The sickling test was positive with metabisulfite. Filter paper electrophoresis revealed type AS hemoglobin. Alkali denaturation showed 0.8 per cent fetal hemoglobin. Frequent urinalysis during this hospitalization revealed grossly bloody urine. Retrograde and intravenous pyelograms were negative. A review of the sections obtained at the time of nephrectomy showed normal kidney parenchyma. Sickling of the red blood cells was noted in the vascular lumina. There were also sickled red blood cells in the ureteral wall associated with a submucosal hemorrhage.

During this patient's last period of hospitalization he became asymptomatic. The hematuria ceased and he was discharged in December, 1953. On follow-up examination he reports no further hematuria.

CASE IV. J. P., a seventeen year old Negro man, was admitted to the Cook County Hospital on May 27, 1953, with a history of painless gross hematuria of two weeks' duration. Cystoscopic study revealed bleeding from the left kidney but the pelvis was found to be normal after pyelogram studies. He was then asymptomatic for a period of three weeks but gross, painless hematuria recurred and he was readmitted to the hospital. There was no history of chills, fever or weight loss. There was a history of tuberculosis in the family. Physical examination revealed a well developed, well nourished, Negro man in no acute distress, and was essentially negative except for small bilateral axillary nodes. Chest x-ray was negative. The Mantoux test was positive in dilutions of 1:1,000 in forty-eight hours. Urine examination revealed gross hematuria. A sickle cell preparation was positive in twenty-four hours by the sealed technic. Hemogram and fragility studies were normal. Bone marrow revealed accelerated erythropoiesis. Paper electrophoresis demonstrated type AS hemoglobin. Fetal hemoglobin by alkali denaturation was 1.2 per cent. Genitourinary study was negative except for the observation of bleeding from the left ureter.

This patient was seen on follow-up examination in September, 1953, and at that time gross hematuria continued. There were no other complaints. No enlargement of the liver or spleen was noted The eyegrounds were negative. Cultures of the urine for acid-fast organisms were negative.

CASE V. C. H. G., a twenty-seven year old Negro man, was first admitted to the Hines V. A. Hospital in 1948 because of a detached retina which occurred while he was in service. At this time a sickle preparation was positive. He was readmitted to the Genitourinary Service in July, 1953, with gross hematuria. Evaluation of the genitourinary tract at that time was considered normal. He was again admitted to the Genitourinary Service in November, 1953, because of a second episode of painless, gross hematuria. Examination revealed a well developed, well nourished Negro man who was in no acute distress. The spleen was palpable 1 cm. below the left costal margin on deep inspiration. The remainder of the examination was essentially normal. Retinitis proliferans of the right eye was found by consultants from the Eye Department; this was thought to be secondary to an old retinal detachment and hemorrhage. Laboratory findings were as follows: Urine cultures for acid-fast bacilli were negative. Retrograde pyelogram revealed filling abnormalities in the right middle calyx suggestive of clots in the pelvis of the kidney. Cystoscopy revealed blood coming from the right ureteral orifice. X-ray of the chest was negative. X-rays of the ribs revealed a general increase in width with slight increase in the trabecular patterns suggestive of the bone changes in sickle cell anemia. The hemogram was normal as was the fragility test. Reticulocyte count was 1.5 per cent. Sickling was positive by the sodium metabisulfite technic. Fetal hemoglobin determination by alkali denaturation technic revealed 1.3 per cent. Filter paper electrophoresis revealed type SC hemoglobin.

The patient was placed at bedrest, had an uneventful recovery and was discharged from the hospital. Subsequent follow-up examinations have not revealed any further evidence of hematuria.

CASE VI. D. P., a forty-one year old, Negro man, was admitted to the Hines V. A. Hospital

on June 28, 1952, with a two-month history of painless and periodic gross hematuria, accompanied by general weakness over the same period of time. He gave no history of prior episodes of hematuria, melena or hematemesis. Physical examination was not remarkable, revealing a moderately well nourished, well developed, Negro man. He was readmitted to the hospital on December 30, 1953, because of gross hematuria, with clots, of three days' duration. Cystoscopy at this time revealed a bloody efflux from the left ureteral orifice. Intravenous pyelograms and renal function tests were normal. Physical examination at this time was essentially negative except for enlargement of the liver which was palpated 2 cm. below the right costal margin. The spleen was not enlarged to palpation. Laboratory findings revealed a normal hemogram. Many target cells were noted in the peripheral smear. Sickling was positive by sodium metabisulfite technic. Fetal hemoglobin was 4 per cent. Filter paper electrophoresis revealed type SC hemoglobin. Phenolsulfonephthalein excretion was 45 per cent in fifteen minutes.

Hematuria persisted until January, 1954, lasting a total period of two weeks, and then stopped abruptly and did not recur. Subsequent follow-up examinations have shown no evidence of hematuria.

CASE VII. J. G., a twenty-five year old Negro man, was admitted to the Hines V. A. Hospital on November 19, 1951, because of painless gross hematuria, with clots, of three days' duration. He complained of nocturia two times a night, but denied chills, fever, weight loss or night sweats. There was no history of venereal disease, urethral instrumentation or tuberculosis. Physical examination revealed a moderately well nourished Negro man. He complained of weakness and light-headedness. The spleen was palpated 6 cm. below the left costal margin and was non-tender, smooth and firm. The liver was not palpable. The remainder of the physical examination was not remarkable.

The hemogram showed the red blood count to be 4,000,000 with 13 gm. of hemoglobin. Platelet count and prothrombin time, serum non-protein nitrogen and blood proteins were normal. Red cell fragility studies were negative, as were the liver function tests. No sickling was noted on direct smear but increased numbers of target cells were found. The patient was cystoscoped and a bloody efflux was noted coming from the left ureteral orifice. Genitourinary studies revealed normal retrograde pyelograms but intravenous pyelograms revealed a filling defect in the left upper calyx suggestive of clots in the pelvis. On December 12, 1951, because of persistent, gross bleeding from the left kidney of four weeks' duration and with evidence of poor filling in the upper left kidney and calyx, left nephrectomy was performed. Grossly the kidney had a smooth surface and was pale yellow in areas. There was considerable perirenal fat. Mucosal erosion of the papillae and petechial hemorrhage were present. Microscopic examination revealed the renal architecture to be preserved. Glomeruli and tubules appeared to be essentially normal. One section of the ureter revealed an erosion of the mucosa, underneath which there was severe hyperemia at the site of bleeding. Sickling of red blood cells was observed in the vessel lumina and in the area of the submucosal hemorrhage. Repeat sickling procedures with sodium metabisulfite revealed a positive sickling test. Filter paper electrophoretic studies demonstrated type SC hemoglobin. Alkali denaturation studies revealed a fetal hemoglobin of 1.3 per cent. The patient was seen once subsequent to his operation at which time hematuria had recurred. This is the only episode of hematuria that he has had.

CASE VIII. D. W., a twenty-nine year old Negro man, was admitted to the Hines V. A. Hospital in December, 1953, with a history of gross hematuria of several days' duration. The hemogram at this time was normal, and a smear for sickling was reported as negative. No further hematologic investigation was made at that time. Intravenous and retrograde pyelograms were normal and cultures of the urine for acid-fast bacilli were negative.

The patient was readmitted in June, 1954, for follow-up examination and the metabisulfite technic for sickling was found to give a positive result. Filter paper electrophoresis revealed type AS hemoglobin. The hemogram was normal as were reticulocyte counts, platelet counts and urinalyses. He has had no further episodes of hematuria.

Summaries of the hematologic and urologic findings in these cases may be found in Tables II and III.

COMMENTS

A review of hospital records (U. S. Veterans Administration Hospital, Hines, Illinois) over a period of four years (1950 through 1953)

revealed seventy-six cases of idiopathic gross hematuria, twenty-two of which were in Negro patients. In the latter group, six were studied by the authors and found to have positive sickling tests. The remaining sixteen were recalled for evaluation and each of these patients had complete genitourinary studies, including intravenous and retrograde pyelograms and urine cultures for acid-fast bacilli. Two patients of this recall group had had nephrectomies because of persistent hematuria. A review of the kidney sections revealed the presence of sickled red blood cells in the lumina of the vessels of one of these cases. Peripheral smear studies for sickle cells prior to his surgery had been reported negative. He has not been available for metabisulfite sickling tests or filter paper electrophoresis studies at this time and for this reason was not included in the present group of patients. Of the remaining fourteen patients, five have been located and re-examined by us and one (Case VIII) has been found to have a sickle cell trait. The remaining four did not exhibit sickling with sodium metabisulfite and electrophoretic patterns revealed homozygous hemoglobin A. Our fourth case was found at the Cook County Hospital.

TABLE II
SUMMARY OF HEMATOLOGIC FINDINGS

Patient	Hemoglobin Type	Fetal Hemoglobin (%)	Sickling		Target Cells	Red Blood Cell Count (million)	Hemoglobin (gm.)	Reticulocytes %	Van den Bergh Test (mg. %)	Splenomegaly	Fragilities	
			Direct Smear	Bisulfite							Initial	Complete
C. A.	AS	1.0	0	+	0	5.0	14	0.1	0.3/0.6	0	36 47	26 30
J. G. B.	AS	1.5	0	+	0	4.7 to 3.8	14	0.2	0.4/0.8	0	40	30
J. L.	AS	0.8	0	+	+	4.5	14	...	0.4/0.9	0	36	22
J. P.	AS	1.2	0	+	0	4.3	.....	...	.....	0	36	24
C. H. G.	SC	1.3	0	+	+++	4.5	13	1.5	0.7/1.3	+	36	24
D. P.	SC	4.0	0	+	+++	2.7 to 4.4	8 to 13	...	0.15/0.5	0	36	24
J. G.	SC	1.3	0	+	++	4.5	15	...	0.15/0.5	++	44	24
D. W.	AS	2.1	0	+	0	4.0	12	0.1	.....	0	..	..

The generally accepted incidence of sickle cell trait in the Negro population is approximately 8 per cent.[19] We believe that the incidence of unexplained hematuria associated with sickle cell trait, which we have noted in six of fourteen patients available for study, is significant and cannot be ascribed to mere coincidence. It would appear that the incidence of hematuria is more frequent with sickle cell trait than in sickle cell anemia. The occurrence of sickle cell anemia being about 0.3 per cent of the Negro population,[19] the age distribution and life expectancy with this disease may account for the difference.

At the beginning of our study, before electrophoresis was available, our cases were considered clinically to be examples of hematuria with sickle cell trait. It was soon noted that several cases were sickle cell hemoglobin-C in type. Clinically, since sickle cell hemoglobin-C disease is associated with symptoms and findings more closely resembling sickle cell anemia than sickle cell trait, we naturally anticipated that most of our cases would fall in the sickle cell hemoglobin-C group. Certainly, hematuria would be expected in the more active form of sickle disease. We believe, therefore, that the occurrence of hematuria in patients with sickle cell trait is perhaps more significant than has been previously realized.

In the three cases of sickle cell hemoglobin-C

disease with hematuria, all had targeting of the red cells, two had splenomegaly and one had evidence of hemolysis and bone changes. None had a history of crisis or exhibited the body build and other features of sickle cell anemia.

All of our cases with sickle cell trait displayed typical electrophoretic patterns, insignificant fetal hemoglobin levels and had no anemia or evidence of hemolysis. Every effort was made by repeated studies to exclude possible genitourinary disease, systemic disease or coagulation defects which could have been responsible for hematuria in these patients. Consequently, we feel justified in linking the hematuria with the presence of sickle cell trait in these patients.

Evaluation of the clinical course of our pa-

TABLE III

SUMMARY OF GENITOURINARY FINDINGS

Patient	Hematuria	Pyelography		Cystoscopy	Cultures	Surgery	Course and Duration
		Intravenous	Retrograde				
C. A.	Gross	Normal	Normal		Negative	None	Onset 1950; has had four episodes of gross hematuria in past four yr.
J. G. B.	Gross	Probable clots in left renal pelvis, 1949; subsequent I.V.P. normal, 1951	Normal	Bleeding from left ureter	Negative	Exploratory laparotomy; normal-appearing kidney and ureter	Onset 1944; has had frequent episodes of gross, painless hematuria clearing spontaneously
J. L.	Gross	Normal, 1948; defect at left ureteropelvic junction, 1949; normal, 1952	Normal, 1948 Normal, 1952	Bleeding from right ureter	Negative	Right nephrectomy, 1949	Onset 1948; frequent episodes of painless bleeding; persistent bleeding until nephrectomy in 1949; sporadic since
J. P.	Gross	Normal	Normal	Bleeding from left kidney	Negative	None	Onset 1953; two episodes of painless gross hematuria
C. H. G.	Gross	Normal	Abnormal filling defects in renal pelvis, thought to be clots on right side	Bleeding from right ureter	Negative	None	Onset, 1948; two episodes of gross painless hematuria in July and Nov., 1953
D. P.	Gross	Normal	Normal	Bleeding from left ureter	Negative	None	Onset 1952; two episodes of gross painless hematuria
J. G.	Gross	Questionable filling defects, suggestive of clots on left	Normal	Bleeding from left ureter	Negative	Left nephrectomy, 1951	Onset 1951; persistent gross hematuria until left nephrectomy; recurrence of hematuria on one occasion since surgery ceased spontaneously
D W.	Gross	Normal	Normal		Negative	None	Onset 1953; one episode of gross, painless hematuria

tients with hematuria revealed a significant absence of pain in association with the episodes of hematuria. All but one patient has had repeated episodes of gross hematuria which subsided spontaneously, including the two patients in whom nephrectomy was performed. When cystoscopy reports were available it was noted that the bleeding was more frequently on the left side, as has been observed by Lund.[10] In the two patients undergoing nephrectomy surgery was performed only after prolonged gross bleeding and it is noteworthy that hematuria recurred in both cases. All other patients were treated primarily with bedrest.

The pathologic findings in our two nephrectomized patients revealed similar changes; namely, submucosal hemorrhage, sickling of the red cells in the vessel lumina with engorgement of these vessels as well as of the capillaries of the glomeruli, and preservation of the normal renal parenchyma. One had ureteral erosion with underlying hyperemia. No thrombosis was seen in any of the microscopic sections reviewed.

The most challenging feature of the association of sickle cell trait with hematuria has been the underlying etiologic mechanism of this syndrome. Since sickle cell trait is not generally accompanied by the usual stigmata of sickle cell anemia, such as crisis, hemolytic anemia, bone changes or unusual body build, we could postulate local tissue insult as the trigger mechanism for vascular injury and subsequent bleeding. Vascular defects of the retina have been described in sickle cell trait by Henry and Chapman.[20]

The kidney is particularly susceptible to the effects of shock, infection and ischemia. Therefore, it is not unreasonable to assume that local changes in the kidney due to such factors might be responsible for the anoxia precipitating the sickling phenomena, with subsequent vascular changes and bleeding. The singular susceptibility of kidney and retina to stress might well account for the unexpected pathologic changes in an otherwise clinically benign condition.

SUMMARY

1. The occurrence of gross hematuria in five cases of sickle cell trait and three cases of sickle cell hemoglobin-C disease is reported. The investigation of these patients included electrophoretic and fetal hemoglobin studies.

2. Some of the previous cases of sickle cell trait reported with symptoms of crisis and/or hematuria might well have been sickle cell hemoglobin-C disease. The literature is reviewed in this connection.

3. The finding of hematuria with sickle cell trait in a significant number of patients is believed to indicate a significant relationship.

4. The singular susceptibility of the kidney to stress associated with sickle cell trait is offered as a possible etiologic mechanism for the vascular defects causing hematuria.

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Adrenal Hormone Therapy in Viral Hepatitis*

IV. The Effect of Gamma Globulin and Oral Cortisone in the Acute Disease

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In preceding studies[1,2] it was shown that the use of ACTH and cortisone resulted in definite improvement in cases of acute viral hepatitis treated with these agents. There was a prompt fall in serum bilirubin and increase in appetite with both drugs. Cortisone in particular brought about a primary recovery period approximately two weeks shorter than did a group of similar controls, and histopathologic studies revealed more rapid progress toward healing. The two principal disadvantages of this form of treatment were a relapse rate of 20 per cent in the treated cases as compared to none in a group of similar controls, and the length of treatment (twenty-eight days) which was prolonged and painful since the intramuscular route was used. Although this therapy had its advantages, it left the patient more vulnerable to relapses, due perhaps to disturbances in virus-host relationships important to immunity.

It was believed that immunity might be bolstered by the concurrent use of gamma globulin, and that treatment might be rendered easier by the oral use of cortisone over a shorter period of time. Values for gamma globulin measured as units of zinc sulfate turbidity had shown a definite decrease with both ACTH and cortisone in previous studies[1,2] which lent further credence to the possibility that additional amounts of this material, given during the course of treatment, might be beneficial. Gamma globulin has been well established as an effective agent in the prevention of infectious hepatitis but its use for the treatment of both acute[3] and chronic[4] viral hepatitis is of questionable value when combined with standard rest and dietary regimens. Its specific application to the problem of enhancing immune mechanisms in patients receiving ACTH or cortisone has not been previously attempted.

MATERIALS AND METHODS

Plan of Study. Three groups of patients were studied, the first two groups consisting of ten patients each, and the last of two patients only, due to time limitations and scarcity of suitable material. These patients, designated as Groups I, II and III, were given oral cortisone and intramuscular gamma globulin according to the following schedule:

Group I received 10 cc. of gamma globulin on the day treatment with cortisone was started, and the same amount one week later. Cortisone was given in amounts of 300 mg. the first day, 200 mg. the second day, 100 mg. the third and fourth day, 30 mg. the fifth, 40 mg. the sixth and 20 mg. the seventh day.

Group II received 30 cc. of gamma globulin at the start of therapy and an additional injection of 20 cc. on the fortieth day of disease. Since all patients were started on this treatment between the tenth and twentieth day of disease, there was little individual variation of the time interval between the first and second injection. Cortisone was administered over a two-week

* From the Hepatitis Center, 98th General Hospital, U. S. Army, Europe. The present study represents a continuation of investigations of viral hepatitis at the Hepatitis Center in Europe which were begun in 1947 under the sponsorship of the Commission on Virus and Rickettseal Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

period, 300 mg. the first day, 200 mg. the second and third days, and 100 mg., 75 mg. and 50 mg. for successive periods of three days each, followed by a final two days of 25 mg. each.

Group III was given gamma globulin injections according to the same schedule as Group II.

probable that the majority of our patients had infectious hepatitis.

Controls. Statistics from control groups collected in previous studies were used[1,2] as they were believed to be recent and sufficiently reliable to be valid.

TABLE I

PRETREATMENT VALUES FOR PREVIOUSLY STUDIED AND PRESENT GROUPS*

Group	No. of Patients	Age	Day of Disease†	Serum Bilirubin	
				Total (mg. %)	One Minute (mg. %)
Previously studied					
ACTH	10	22.9	14.6	10.9	5.8
Alternate control*	10	22.5	13.9	9.6	4.9
Prior control	100	24.1	14.0	9.1	4.8
Gamma globulin + cortisone					
Group I	10	23.6	12.3	12.0	6.1
Group II	10	22.3	15.1	11.3	5.1
Group III	2	22.0	16.0	9.0	4.9

* The alternate control group represent patients given placebo injections in alternation with patients receiving ACTH in a previously reported study.[1]

† Day of disease indicates day when hormonal therapy or control injections were started and day of admission laboratory studies for the prior control group.

Cortisone was administered over a three-week period on a somewhat higher average dosage schedule as follows: 300 mg. the first three days, 200 mg. the next four days, 150 mg. for five days, 100 mg. for five days, and 75 mg. and 50 mg. for two days each.

Criteria for All Groups. (1) Uncomplicated viral hepatitis as based on typical history, physical and laboratory findings; (2) demonstrably rising total serum bilirubin over 5.00 mg. per cent prior to the start of therapy; (3) onset of disease not more than twenty or less than ten days previous to therapy; (4) absence of contraindications to cortisone therapy; and (5) voluntary consent for liver biopsy following treatment. The period of ten to twenty days after onset of disease was chosen for institution of hormone therapy rather than an earlier period because our former experience[1,2] suggested that such delay might reduce the relapse rate and in addition it eliminated those patients in whom rapid recovery occurred without hormone treatment. It was not possible to determine whether the type of viral hepatitis encountered was due to virus A or virus B, although it is

Biopsies of the Liver. These were performed between the thirty-fifth and fortieth days of illness. None were done prior to therapy since it was thought that previous material was ample for comparison and that study following therapy at this time interval would serve to establish the diagnosis as well as effects of therapy and would be comparable in timing to previous studies in which controls were used. Adequate specimens were obtained in all cases and no complications were encountered.

Observations. Clinical observations were recorded on a standard form and laboratory procedures including complete liver function studies weekly or oftener followed the pattern of preceding studies,[1,2] with the exception that electrocardiograms were not performed routinely.

A high protein, 4,200 calorie low salt diet and absolute bedrest were prescribed as before.[1,2]

PRETREATMENT STUDIES

The status of patients immediately prior to institution of oral cortisone-gamma globulin therapy given in Table I is compared with similar pretreatment data for previous patients in a

similar period of illness (second ten days after onset) who had been given ACTH or served as controls.[1] The age of the patients and duration of disease at the start of therapy or the control period were quite similar for all groups. The total serum bilirubin of Groups I and II of the present study were 2 to 3 mg. per cent higher, however, than the control groups. This has some bearing on the results to be presented since the intensity of jaundice is one expression of the severity of disease and of the expected duration of the hepatitis. Not included in Table I are previous patients started on ACTH or cortisone therapy in the first ten days of illness.

TABLE II*

COMPARISON OF RESULTS IN GAMMA GLOBULIN–CORTISONE STUDY GROUPS WITH PREVIOUSLY TREATED AND CONTROL GROUPS

Groups	No. of Cases	Duration of Hormone Therapy (days)	Day of Disease when Test Returned to Normal		Relapses in Group
			Total Serum Bilirubin	BSP Retention (10% or less)	
Previously treated					
ACTH	10	28	44.1	55.0	0
Alternate control	10	0	55.1	57.3	0
Prior control	100	0	55.1	59.4	0
Gamma globulin + cortisone					
Group I	10	7	49.9	60.3	2
Group II	10	14	46.9	53.1	2
Group III	2	21	32.5	46.5	0

* This table includes only patients started on therapy or control period ten to twenty days after onset of symptoms. Those previously placed on the program in the first ten days of illness using ACTH[1] or cortisone[2] are omitted.

RESULTS OF THERAPY

Patients treated with oral cortisone had a rapid and often dramatic fall in serum bilirubin within the first few days of therapy and this was usually accompanied by an increase in appetite and well-being. The effects were similar to those previously observed with parenteral cortisone or ACTH.

The duration of illness measured by the day of disease on which the total serum bilirubin (TSB) reached normal (1.0 mg./100 cc.) and the bromsulfalein retention (BSP) reached 10 per cent (forty-five minute sample) or less is compared in Table II with results of our earlier studies. Patients given oral cortisone for one or two weeks did not recover much more rapidly in terms of these criteria than control patients despite their good initial response. That this may be related to the duration of hormone therapy is suggested by the shorter duration of illness in the two subjects receiving three weeks of therapy in whom results were comparable to those of parenteral cortisone given for four weeks in which the TSB and BSP became normal on the thirty-first and forty-second day of illness, respectively.[3]

One other factor bears on these results. The experience at this Center with more than 5,000 cases of viral hepatitis has emphasized that although the duration of disease is unpredictable in an individual patient, good correlation exists in large groups of patients between the height of bilirubinemia and the total duration of jaundice.[5] The serum bilirubin at the start of therapy in patients in the present study was sufficiently higher than in control patients to merit analysis from this standpoint. This has been done in Figure 1 in which data are included on ten patients started on ACTH at a comparable time after onset of illness. The results indicate that the total period of bilirubinemia was proportionally shortened by both oral cortisone and parenteral ACTH therapy as compared to control groups. We hold no brief that this effect necessarily indicates recovery from hepatitis but wish to emphasize that adrenal hormone therapy significantly influences the disappear-

ance of jaundice. Just how this occurs is unknown. It might operate through extrahepatic mechanisms such as increasing renal excretion or inhibiting a hypothetic hemolytic factor. A direct hepatic action is far more likely, however, The more rapid histologic healing in liver

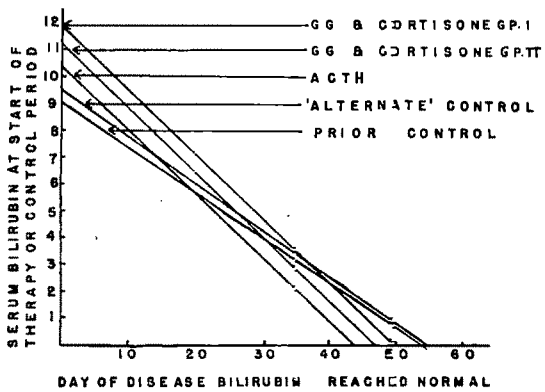


FIG. 1. Rate of return of total serum bilirubin to normal. In this chart only patients are included who were started on hormonal therapy or control period ten to twenty days after onset of illness.

biopsies from patients given cortisone over a four-week period[2] supports this.

EFFECT OF CORTISONE ON LIVER FUNCTION

Serum Bilirubin. As already indicated, all twenty-two patients treated with cortisone showed typical prompt initial drops in total serum bilirubin values similar to those noted previously for this type of therapy[1,2] There was considerable irregularity in the "leveling out" phase after the initial drop, especially in Group II. In general, results were comparable to previous studies.

Bromsulfalein Excretion. Values for this test were quite similar to control groups except in the two patients of Group III who showed a better response after receiving cortisone therapy for twenty-one days.

Other Liver Function Tests. Since such tests as thymol turbidity, cephalin cholesterol flocculation, alkaline phosphatase and cholesterol ester per cent showed little variation from controls previously,[1] and since individual variations appeared to be lacking during this study, the values were not summarized. Gamma globulin, as shown by zinc sulfate turbidity values, showed wide variations which precluded accurate graphing. There was no instance however, of elevation of values following injection of human serum globulin and, in general, values showed mild to moderate lowering during the course of treatment, following previously noted trends.

RELAPSES

The term 'relapse" has been defined for the purposes of these studies as the recurrence or recrudescence of clinical signs and symptoms of hepatitis confirmed by definite changes in liver function after an initial period of improvement.[1] By these criteria gamma globulin did not prevent relapses. Two occurred in Group I (ten patients), two in Group II (ten patients) and none in Group III (two patients). This relapse rate of approximately 20 per cent is similar to that of patients treated with ACTH or cortisone over a longer period but not in conjunction with gamma globulin.[1,2,6] All four relapses of the present series occurred at the time cortisone was discontinued There were no "late" relapses. In four patients the relapse was characterized by a second peak of bilirubinemia higher than the first and by anorexia, malaise and abdominal pain. Relapses of this type as well as some encountered either during therapy or some time after discontinuance of therapy have been previously reported.[1,2,6]

Two other cases of the present group did not fulfil our criteria for relapse but their courses were not smooth and the response to therapy was not ideal. In the first an asymptomatic increase in bilirubinemia occurred at the cessation of therapy. In the second, erratic increases in bilirubinemia were seen during cortisone therapy. These responses, while not true relapses, probably delayed convalescence.

None of the relapses were retreated with ACTH or cortisone so that the course of disease could be observed under such circumstances. It is known that they do, in fact, respond satisfactorily to reinstitution of therapy.[6–8]

SIDE EFFECTS

Response to cortisone in the three groups is shown in Table III. The high per cent of glycosuria is consistent with previously observed disturbances in carbohydrate metabolism, although hyperglycemia was not noted. Eosinophil response was initially good in a high per cent, although later rise was noted in most patients under treatment.

CHANGES IN LIVER HISTOPATHOLOGY

The biopsy material was processed in the manner outlined in other communications.[1,2] All slides were examined as unknowns. The group of control cases was the same as before.[1,2] The biopsies were obtained from thirty-one to fifty-six days after the onset of the disease: all twenty-two showed evidence of a diffuse hepatitis consistent with the diagnosis of viral hepatitis.

TABLE III
SIDE EFFECTS OF CORTISONE AND GAMMA GLOBULIN THERAPY

	Incidence (%)		
	Group I (10 Cases)	Group II (10 Cases)	Group III (2 Cases)
Glycosuria	70	100	100
Euphoria	10	30	50
Rounding of face	30	100	100
Eosinopenia	90	90	100
Acne	20	50	100
Hyperglycemia	0	0	0
Hypertension (over 140/90)	10	0	0
Edema	10	0	0

There was good correlation between the clinical course and the histologic findings. Corresponding to the interval between the onset of clinical relapse and the time the liver biopsy was obtained, three cases showed evidence of hepatitis in an acute, active phase. In the remaining nineteen cases the clinical course was favorable in twelve, with steady progression toward cure and without any evidence of chemical or clinical recurrences or recrudescences. The section of liver in eleven of these twelve cases showed evidence of a subsiding hepatitis with minimal to slight activity, findings fully within the range expected at the time of biopsy. Only one showed evidence of a more marked, moderately severe activity. Six of seven other cases in which there had been some slight secondary rises of serum bilirubin or more prolonged abnormality of liver function tests showed greater severity of histologic lesions which, however, were still considered to be in the upper range expected for this stage of the disease process.

Excluding the patients who relapsed, there was no significant difference between the liver biopsies of patients given cortisone and those untreated. Similarly, the variation between biopsies in Groups I, II and III was not greater than the individual variations within the group itself. Fatty metamorphosis of liver cells was never conspicuous in biopsies from patients who had been treated and did not exceed that of control patients. These results are in contrast with those observed when parenteral cortisone was administered for four weeks.[2] Here the healing process was uniformly more rapid than in controls but was accompanied by fatty metamorphosis. Such differences are probably the consequence of more prolonged hormonal therapy.

DISCUSSION

Frequent relapses have been a major disadvantage of treatment of viral hepatitis with adrenal hormones and may have resulted from hormone-induced disturbances in the development of immunity.[1,2] One of the primary purposes of the present study was to determine if the administration of gamma globulin could enhance the level of immunity sufficiently to prevent relapse. No such protection occurred, as evidenced by a 20 per cent relapse rate similar to that in ACTH- or cortisone-treated patients not receiving gamma globulin.

Infectious hepatitis may be effectively prevented by gamma globulin injections given after proved exposure to this disease but serum hepatitis is not prevented in this way even when the gamma globulin is prepared from the blood of volunteers convalescent from known serum hepatitis.[9] These observations may bear on the present study. Gamma globulin would not be expected to enhance immunity to serum hepatitis since it does not prevent the disease, yet it might be effective in infectious hepatitis. Possibly the relapses encountered in this investigation occurred only in patients with serum hepatitis. This seems unlikely but since it has not been possible to differentiate infectious from serum hepatitis on clinical or epidemiologic grounds in this study[10] this question cannot be answered directly.

Oral cortisone appears to be fully as effective as intramuscular cortisone in alleviating symptoms, stimulating appetite and enhancing the disappearance of jaundice. The best results have been obtained when cortisone is given for three or preferably four weeks. There is evidence from our previous study that even histologic healing of the liver may be accelerated under such management.[2]

Despite these results we strongly believe that neither cortisone nor ACTH has any place in the routine management of viral hepatitis. The beneficial effects are more than offset by the frequency and severity of relapses. Furthermore when the average patient with hepatitis is put to bed and given a nutritious diet, recovery is usually uneventful and without sequelae. Adrenal hormone therapy does not shorten the course of total illness sufficiently to merit the risk of relapse, the expense of treatment and the possibility of unpleasant side effects.

Two situations deserve consideration for cortisone therapy. The first of these is fulminant hepatitis with coma. In over 5,500 cases of viral hepatitis studied at the Hepatitis Center, approximately sixteen patients developed definite coma.[5,6] Of these, five received ACTH, two received cortisone and the rest were treated with supportive therapy only. Of the sixteen patients only one recovered and she received massive cortisone therapy (1 gm. daily).[11,12] One other patient with very severe and rapidly progressive hepatitis in pre-coma also recovered while receiving large doses of cortisone.[6] Since leaving the Hepatitis Center, the authors have treated two other definitely comatose hepatitis patients with massive cortisone therapy, one of whom recovered.* Ducci and Katz have reported the recovery of five comatose hepatitis patients given similar therapy.[13,14] Coma due to viral hepatitis therefore represents a clear-cut indication for a trial of cortisone therapy in very large doses.

The other situation in which cortisone merits trial is the severe, rapidly progressive case of marked jaundice (over 15.0 mg. per cent) in which anorexia and vomiting have not responded to intensive conservative therapy with intravenous glucose. Our experience with ACTH in this situation has been unfavorable, attended as it was by frequent relapses of major and minor severity and occasional severe side effects[6] but recent trials with cortisone under these conditions have been more promising. The attendant danger of relapse is ever present, however, and one must reinstitute therapy promptly if this occurs. Cortisone should probably be continued for a period of not less than three to four weeks, preferably until the serum bilirubin reaches normal, and then discontinued gradually. The advantages and disadvantages of such management should be weighed carefully against the seriousness of the patient's condition.

* The fatal case is included in the references,[6] the other is unreported.

SUMMARY

1. Twenty-two patients with acute viral hepatitis were given oral cortisone in conjunction with the administration of large amounts of gamma globulin intramuscularly at the beginning and end of hormonal therapy.

2. Gamma globulin failed to prevent relapses: four occurred, an incidence similar to that in ACTH- or cortisone-treated subjects without conjunct gamma globulin therapy.

3. Oral cortisone produced a rapid initial drop in bilirubinemia but failed to influence significantly the total duration of illness except in two patients receiving therapy over a three-week period.

4. Even under ideal circumstances cortisone does not shorten the duration of disease sufficiently, in our opinion, to run the increased risk of relapse, and thus is not recommended for the routine treatment of viral hepatitis. It may have a place in the treatment of severe and progressive viral hepatitis and certainly deserves further trial in fulminant hepatitis with coma.

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Seminar on Carbohydrate Metabolism

Experimental Diabetes and Its Relation to Diabetes Mellitus

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"There is, however, a Nemesis which waits upon those who deliberately avoid avenues of knowledge." (*Alfred North Whitehead, "Science and the Modern World."*)

EXPERIMENTAL diabetes refers to the production of hyperglycemia, glycosuria and faulty carbohydrate metabolism in animals. This approach to the problems of diabetes has made possible the discovery of insulin and has enabled physicians to recognize phenomena related to diabetes mellitus which could not have been as readily revealed by the study of man. A few such phenomena will be chosen for comment. Some of these, such as insulin, were formerly hypotheses but have now been demonstrated by subsequent investigation; others provide the working hypotheses of today. The subject of experimental diabetes has been well reviewed by Warren and Le Compte[1] whose chapter provides much of the background for these comments.

ESTABLISHED PHENOMENA (INITIALLY MERE POSSIBILITIES)

Insulin. The existence in the pancreas of a factor which controls carbohydrate metabolism was clearly demonstrated by von Mering and Minkowski in 1889. Thirty-three years later this long recognized possibility was realized in the discovery of insulin by Banting and Best. The fulfillment of this early possibility is cited before considering the problems of today.

Overproduction. In the overproduction theory of diabetes[2] it was assumed that there was no diminution of the utilization of blood sugar by the tissues but that the supply of sugar to the blood from the liver was so excessive that continued normal utilization could no longer dispose of it. Early students of diabetes recognized the possibility that all or part of the picture of diabetes might be due to the excessive catabolism of protein and fat. In both normal and depancreatized animals the early studies on hepatectomy and the modern investigations on the effects of hypophysectomy or adrenalectomy have shown that the secretion of glucose into the blood and the excretion of nitrogen are reduced. When Houssay removed the pancreas in hypophysectomized dogs and observed that the excretion of glucose, nitrogen and ketones were less than the amounts expected after pancreatectomy, he showed that excessive gluconeogenesis (overproduction) was a component of pancreatic diabetes. Later, Young produced diabetes by administration of pituitary extract and left no doubt as to the importance of the anterior pituitary in diabetes.

These discoveries still leave many problems for the future. How frequently does an excessive secretion of pituitary or adrenal hormones act to cause or to aggravate human diabetes? Stress of any kind accelerates gluconeogenesis which is at least in part due to excessive activity of the adrenal cortex.[3–5] The exacerbations of diabetes which accompany infection and certain other diseases are better understood because we now know something about the mechanism of overproduction.

Before the days of antibiotics, when the pancreas was removed the incisions often developed rapidly progressive abscesses. After pancreatectomy in hypophysectomized animals such abscesses healed completely, even in the total absence of insulin and in the presence of hyperglycemia. However, in the animal which had undergone two operations (Houssay) there is no exaggerated breakdown of protein and fat and there is no ketonuria. Since protein and fat breakdown usually occur together, the avoidance of marked ketonuria in man after surgery

might serve as a simple guide in controlling catabolism sufficiently to permit good healing.

Underutilization. From early observations it was postulated that all or some of the phenomena of diabetes might be due to failure to utilize glucose in the normal manner. Recent studies with isotopically labelled substances have resolved this problem as far as the overall or net metabolism of glucose is concerned.[6,7] After total pancreatectomy there is impaired utilization of glucose in the dog[7] and this has been confirmed in man.[8] This impaired utilization is not complete and, in fact, does not differ greatly in degree from that of the fasted normal animal, yet it represents only a small proportion of the utilization of glucose by the normal fed animal. When insulin alone is deficient, the oxidation of glucose and fat synthesis seem to be the metabolic pathways which are particularly affected, but information about these individual pathways requires further study.

In any case the deficiency of insulin is accompanied both by underutilization of glucose and by exaggerated catabolism in varying degrees. To this extent both underutilization and overproduction have been clarified by later observations.

POSSIBILITIES INFLUENCING CURRENT THINKING

Pathways of Carbohydrate Metabolism. In an earlier article of this series Stetten and Topper[9] outlined the knowledge of the pathways of carbohydrate metabolism. Experimental diabetes has contributed much to our knowledge of the biochemistry of carbohydrate, fat and protein.

Mechanism of the Action of Insulin. This is one of the major problems now before us and is reviewed in this series by Stadie.[10] Experimental diabetes continues to be one of the essential methods of the investigator of insulin and it makes possible the broad physiologic picture of diabetes into which the exact information about the action of insulin will eventually be fitted.

The Islands of Langerhans. In 1938 Warren listed toxic injury of the islands of Langerhans as a possible cause of diabetes, although no such cause was known at that time. When Dunn, Sheehan and McLetchie[11] in 1943 described the injury of the islands produced by alloxan, this form of experimental diabetes gave the first factual support to the possibility of toxic injury. Since 1943 other substances which damage the islets have been described.[12] None of these has been identified as a cause of diabetes in man. On hydrolysis of human urine Paley et al.[13] found oxomalonic acid which might have come from alloxan although its exact source has not been ascertained.

Heredity in Diabetes. An increased incidence of the disease in the families of diabetics has brought about recognition of the heredity factor. This and related observations have been reviewed by Joslin et al.[14] Cole, Harned and Keeler[15] found that inheritance in rats of a decreased tolerance for glucose was not a simple recessive characteristic. More recently the "hereditary, obese-hyperglycemic syndrome" in mice has been studied by Mayer.[16] In these mice the islands of Langerhans are increased in size and number; the mice are resistant to insulin and unduly sensitive to the diabetogenic action of growth hormone. Human heredity is so complex that it is often difficult to distinguish genetic, developmental and environmental factors. The breeding of diabetic strains of rats and mice indicates that a genetic factor in diabetes is possible. Knowledge of the various ways in which the disorder is inherited in animals may help in the examination of the problem in man.

Obesity and Diabetes. The regulation of appetite and the various causes and types of experimental obesity have been reviewed by Mayer.[16] The hereditary diabetes of animals already referred to[15,16] is associated with obesity. This condition differs from the situation in the juvenile diabetic who is rarely obese but agrees with the association of events in elderly diabetics. In addition, differences have been observed between the various types of experimental obesity. Marshall and Mayer[17] report that "The food intake of gold thioglucose obese mice is 75–100 per cent greater than that of non-obese animals, the oxygen consumption is greater than that of non-obese animals and the spontaneous physical activity of gold thioglucose mice is similar to that of non-obese animals. These findings contrast with results obtained from genetically obese mice, which are characterized by slight relative hyperphagia, low oxygen consumption, and depressed physical activity. This comparison emphasizes the multiple etiology of obesity." Such metabolic differences in obesity should be thought-provoking to physicians.

Undernutrition in Treatment. Allen[18] on both experimental and clinical grounds emphasized the earlier observations on the value of undernutrition in the control of many of the mani-

festations of diabetes. This is still recognized. Although much has been written about the relation of the vascular complications to the control of the metabolic disorder, there is little information about the influence which the control of obesity might have on these complications. Such work requires many years of clinical observation and is not likely to be solved in the laboratory. Even so, experimental diabetes gives some food for thought. Duff and McMillan[19] found less experimental atherosclerosis in diabetic than in normal rabbits. They said, "gain or loss of body weight during the (cholesterol) feeding period exercised no determining influence." In contrast Firstbrook[20] found a high net correlation between relative weight gain and the severity of experimental atherosclerosis.

Dublin and Marks[21] have reviewed the mortality of obese persons and have said that "A feature of the study is the experience of those overweights who brought down their weight. . . . This is perhaps the best evidence to date that there is long range benefit from weight reduction." The extent to which this improved mortality was due to the prevention of vascular damage was not determined and Liebow et al.[22] concluded that obesity did not influence arteriosclerotic heart disease in diabetes. The specific diabetic changes such as retinopathy and glomerulosclerosis do not develop in obese persons without diabetes; these changes develop in many thin diabetics, but the possibility that control of obesity might be a useful adjunct in some situations still seems to deserve study.

Appraisal of the Severity of Diabetes. Some knowledge of the fundamental observations in the experimental laboratory is required. Although the experimental facts concerned with this appraisal are not new, their meaning is often overlooked by physicians. Various criteria of the severity of diabetes are needed, depending upon the circumstances. Figure 1 graphically represents some of the measurements of the metabolic disturbance in animal and man.

In very mild diabetes only the abnormality of the glucose tolerance test will determine the presence of the disorder. There is no more accurate quantitation at this stage. In a few mild diabetics the blood sugar, under standard conditions such as fasting overnight, may range between normal and the renal threshold of 180 mg. per 100 ml. In these few persons the level of blood sugar probably records differences in the severity of diabetes. When the blood sugar exceeds the renal threshold, it ceases to give information about the severity of the disease. Experimentally pancreatectomy has been combined with administration of phlorhizin.[23] Under these conditions the blood sugar of the depancreatized animals remained at 70 to 80 mg. per 100 ml. but the amount of sugar in the urine, the survival time and all other manifestations of diabetes were unaltered. In Figure 1 the schematic daily blood sugars of moderate and severe (depancreatized) diabetes are merely values above the renal threshold.

The very high values of blood sugar encountered in diabetic coma in animals or man are usually associated with circulatory shock in which the kidney can no longer clear the blood of glucose in its usual fashion. In phlorhizinized-depancreatized cats[23] the blood sugar rose to high levels (for example, 700 mg./100 ml.) before death, and in the series of patients reported by Martin and Wertman[24] all of those with blood sugar levels above 800 mg./100 ml. were in shock. The author regards a very high blood sugar in diabetic acidosis as a sign or warning of circulatory shock.

About 50 per cent of diabetics on a maintenance diet need insulin to control glycosuria. On the chart this group is called MODERATE in severity even though it extends to the point at which 100 per cent of dietary glucose is excreted. In such diabetics (animal or human) the proportion (that is, per cent) of dietary carbohydrate which is excreted in the urine provides a good index of the severity of the disorder. For simplicity one may use all of the dietary carbohydrate and half of the protein of the diet in calculating the so-called available glucose. (The exact D N ratio is uncertain but a commonly observed factor of this order of magnitude serves the purpose.) The proportion of the available carbohydrate excreted remains fairly constant over a wide range of dietary intake. Dohan et al.[25] described this in a study of pituitary diabetes in dogs and Sturtevant and Fuller[26] have emphasized its usefulness in alloxan diabetic rats.

When a physician is asked, "How severely diabetic is Mrs. X?" he will usually reply, "Severe. She takes 70 units of insulin daily." The dose of insulin needed to control diabetes on a maintenance diet is a useful criterion of the severity of the disease, but it is only one criterion. In Figure 1 the daily dose of insulin is charted as a broken line which parallels the severity of the

disease as measured by the amount of glucose excreted. The end of this line does not touch the baseline because insulin will probably not be used in mild diabetes.

Both the dose of insulin and the proportion of dietary glucose excreted are subject to fairly parallel lines of the present drawing. Nevertheless when gross complicating factors are excluded both of these measurements afford an approximate but serviceable gradation of this segment of the diabetic state.

The most severe diabetes is that due to total

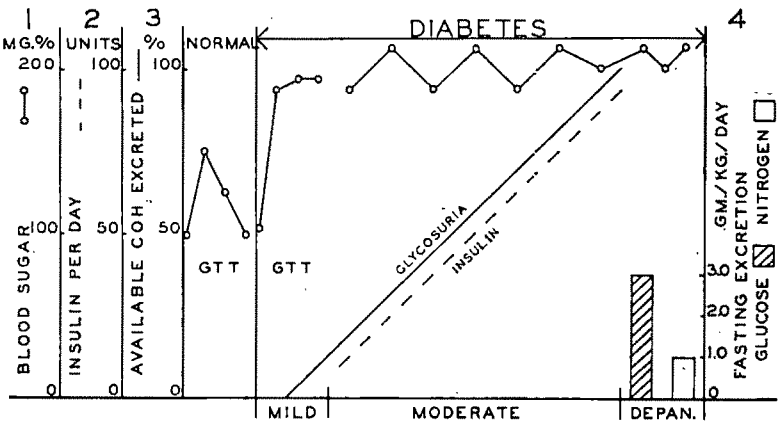


FIG. 1. Four measures of the severity of diabetes (schematic). The units of measurement are numbered 1 to 4 at the top of the chart. The purpose of this diagram is to emphasize the fact that different criteria of the severity of diabetes are required for MILD, MODERATE and total pancreatic (DEPAN.) diabetes. This may be further emphasized by noting what is not charted. Thus the normal person has no glycosuria and needs no insulin administered; the glucose tolerance test (G.T.T.) gives normal results. In mild diabetes the G.T.T. gives abnormal results and glycosuria may or may not be present; hence the solid line that shows the proportion of dietary glucose excreted does not include all of the zone marked MILD. Insulin is not needed in mild diabetes so that the broken line begins still further up the scale of glycosuria. A given point on the parallel lines for glucose excretion and insulin dosage will be raised by infection or lowered by adrenal insufficiency, to cite two examples only.

The zone of MODERATE diabetes is here extended to the point where 100 per cent of available glucose is excreted. In dogs ketonuria appears when 80 to 90 per cent of the available glucose is excreted. Ketonuria is omitted because its appearance and amount are even more irregular than the crude quantification of the proportion of dietary glucose excreted or the dose of insulin. In moderately severe diabetes the level of blood sugar is represented as being constantly above the renal threshold. Hence the blood sugar tells nothing of the severity of diabetes, once it exceeds the renal threshold. The very high levels of blood sugar which are observed in diabetic coma are the result of circulatory-renal failure (shock) and are a secondary effect of severe diabetes but they do not measure the metabolic defect. Therefore, such figures have been omitted. Finally, severe diabetes in the human usually has little or no glycosuria during fasting (compare with Table 1) so that the excretion of glucose and nitrogen during fasting is not charted except under DEPAN. Due to the defective absorption of food after pancreatectomy the maintenance requirement for insulin cannot be compared to that for diabetes in animals or men whose digestive enzymes are functioning. For this reason insulin dosage is not charted in the DEPAN. zone.

wide variations with complicating circumstances. One of the most important of these is infection, which will increase glycosuria and insulin requirements. In contrast the reduced glycosuria of the Houssay animal or of the patient with adrenal insufficiency and diabetes would be charted here by lowering a given point on both pancreatectomy. (Fig. 1.) This is so because of the absence of insulin and the violent metabolic reaction thereto. Under these conditions the dog and cat excrete about 3.0 gm. of glucose and 1.0 gm. nitrogen per kg. per day during fasting, so these amounts have been used in the diagram. Since most diabetic patients become sugar-free

on fasting, such units of measurement are not used elsewhere in Figure 1.

Table 1 reviews some frequently cited data about the response to pancreatectomy in different species as measured by the fasting metabolism. The observations of Geyelin and Du Bois[27]

TABLE 1
FASTING METABOLISM IN DIABETES

Species or Subject	Urine				D:N	Survival (days)
	Glucose (gm./kg./day)		Nitrogen (gm./kg./day)			
	Normal	Depancreatized	Normal	Depancreatized		
Cat	0	3.2	0.7	1.4	2.3	4
Dog	0	2.8	0.5	1.0	2.8	10
Goat	0	0.12	0.13	0.38	0.3	44
*C. K.[27]						
Dec. 1915	0	1.3*	.....	0.41*	3.1	..
Jan. 1916	0	0	0.17†	0.17*	0	..
F. W.[29]	0	....	.....	.....	2.4	6

* Severely diabetic, not depancreatized.
† Normal man; average of first nine days of fasting (calculated from F. G. Benedict[30]).

on a severely diabetic man have been added for comparison. In December 1915, this patient (C. K.) had mild acidosis from which he recovered as a result of undernutrition and drainage of a carbuncle; this treatment would not save the depancreatized dog. The increased excretion of nitrogen during this period ("higher than any hitherto observed in man") resembles the increase seen in animals after pancreatectomy, but the patient's spontaneous recovery makes one hesitate to assume that these figures represent an absence of insulin. He ultimately tolerated 160 gm. of carbohydrate and 3,500 calories daily. According to Table 1, the wasting of protein (nitrogen) during fasting is a finding common to all kinds of depancreatized animals. This is so regardless of variations in the amount of glycosuria, the period of survival, the presence or absence of ketonuria and other factors.

In connection with the problem of measuring the severity of diabetes, two references may be cited. First, Lusk[28] reported a man with mild diabetes who could take 40 gm. of carbohydrate daily without glycosuria. "He became completely diabetic with a urinary D:N of 3.84:1 on the second day after administering a diet containing considerable quantities of protein and fat. Subsequently, virtual starvation led in 60 hours to the disappearance of sugar in the urine. It was thus proved that a potential diabetic could be transformed into a completely diabetic individual. . . . " The fasting D:N of patient C. K. in January, 1916 (Table 1) and the contrasting D:N of Ricketts'[29] patient suggest that most of the patients reported by Lusk were not completely diabetic. What indeed is "complete" or "total" diabetes? It is the fulfillment of some criterion adopted by the particular investigator. The author has tried to avoid such terms and can only say that the most severe form of diabetes appears to be that which meets appropriate criteria of the totally depancreatized animal of the same species. In a study of dogs with pituitary diabetes,[25] three animals were encountered in which the fasting excretion of glucose and nitrogen equaled that of depancreatized dogs, although in pituitary diabetic dogs the pancreas was surgically untouched.

Second, Marsky[31,32] has ably used the fasting metabolism to elucidate the fact that diabetes of the depancreatized dog is more severe than that of the aloxanized dog although the latter requires more insulin when fed. The desirability of obtaining information of this sort about man is apparent from the references already cited and from the fact that these spaces for depancreatized man remain blank in Table 1.

In Figure 1 insulin dosage refers to the amount per day which is needed to control diabetes on a maintenance diet. The insulin requirements of the depancreatized man, often about 40 units daily, cannot be compared to the requirements of other diabetes because of the variable but grossly defective absorption of protein and fat after this operation, even when the best available exocrine therapy is given.[29] Most uncomplicated diabetics need no insulin during fasting if one may judge from the old records and, as the author knows of no data on the insulin requirement of the fasting depancreatized man, no figures are presented for comparison. Depancreatized dogs, which needed 40 units of insulin daily when fed, were well controlled on four units daily during fasting.

Another type of study on food intake and insulin requirement has been performed by Singh.[33] This included six patients with severe diabetes who originally required 80 to 120 units of insulin daily. After six weeks on low-fat optimal caloric diets containing 20 to 30 gm.

of fat these severely diabetic patients could be controlled on 20 to 40 units daily. Since the absolute amount of fat fed to these patients coincides in a general way with the amount absorbed by depancreatized men (compare studies cited in Ref. 29), the achievement of a similar insulin dose in both instances seems noteworthy. Such experiences prepare one to find a lowered insulin requirement with the defective food absorption which follows pancreatectomy. The author has not yet heard of a depancreatized person becoming obese.

Only passing reference will be made to certain other criteria of the severity of diabetes. The period of survival after pancreatectomy is listed in Table 1 and the only clinical observation[29] makes human pancreatic diabetes comparable to that of the dog or cat in this respect. Another criterion is the rate of weight loss after pancreatectomy. This is greatly influenced by the catabolic reaction to the removal of insulin, as shown by simple laboratory findings. Thus depancreatized cats lose 300 to 400 gm. in the four days of survival; depancreatized-hypophysectomized cats lose the same amount of weight in two months. Both lose weight because they lack insulin, but when the pituitary and adrenal cortical catabolic hormones are in action this process is violently accelerated. In a slightly different way the same thing has been observed in man. Thus a fasting man[28] lost an average of 0.57 kg. per day during the first ten days. Patient F. W.[29] lost 1.5 kg. daily in the first three days of a period without insulin, or almost three times the rate of the fasting normal man.

In conclusion: (1) both experimental diabetes and clinical experience make it clear that multiple criteria of the severity of diabetes are required under different conditions; (2) choice of these criteria has been difficult in the past and may well be revised by future experience; and (3) there is basic information still to be learned about human diabetes by applying experimental criteria to depancreatized man.

Table 1 illustrates the response to pancreatectomy in different species. Other species differences exist, three of which will be mentioned.

1. The response to so-called diabetogenic hormones varies widely with the species. This is presumably an extra-pancreatic difference in the animals and may be illustrated by the following well known laboratory findings.

The rat develops hyperglycemia and glycosuria when treated with corticotrophin or cortisone, both of which are ineffective in dogs and cats. On the other hand pituitary growth hormone is ineffective in rats but is a powerful diabetogenic agent in cats and dogs. Unpublished observations (Lukens) on the production of diabetes in cats with some new synthetic cortisone derivatives seem to indicate that this is the result of the degree or intensity of hormonal action as well as of the susceptibility of a particular species.

2. When hyperglycemia and glycosuria are produced by any means, the behavior of the islands of Langerhans differs greatly in different species. In the rat the islands do not suffer any appreciable degenerative changes. Even when they are overwhelmed by a diabetogenic hormone the diabetes ceases when the hormone is discontinued. The rat also has considerable capacity for islet hyperplasia. Contrariwise, in the cat and dog partial pancreatectomy, pituitary extract (growth hormone) or massive injections of glucose lead to degenerative changes and atrophy of the beta cells of the islands, so that permanent diabetes may ensue. Relatively little capacity for islet hyperplasia exists in these animals.

3. Even in the same species the islands of Langerhans may behave differently at different times. The normal cat given massive injections of glucose develops degranulation, hydropic degeneration and atrophy of the islands. After the administered glucose is discontinued the animal may have mild diabetes for an indefinite period. At this time the same degree of hyperglycemia and glycosuria leads to no progression in the severity of the diabetes. No explanation for these observations exists but the facts must be reckoned with as one attempts to learn more about the islands of Langerhans in human diabetes.

The different behavior of the islands in the rat and the dog cannot be foretold by their essentially similar appearance histologically. Little is known of the behavior of the islands in man and such laboratory experiences should alert us to look for these and other possibilities in the islands in human subjects.

Transient Causative Factors. Experimental diabetes has demonstrated that a causative agent of diabetes may have a transient existence and may not be observed in a diabetic animal studied at a later date. We do not know the extent to which this may apply to human diabetes and it has been said that the cause or causes of diabetes

may have to be determined before the person studied actually becomes diabetic.

Experimental studies of the influence of diet on the islands of Langerhans[34] combined with further observations on obesity as a precursor of diabetes in man[14,21] should yield more knowledge of the diabetic state. The development of diabetes years after women have been delivered of large babies[35] may provide another source of information about the conditions that precede the overt metabolic disorder. Meanwhile the facts that (1) no alloxan is present five minutes or so after its injection and (2) no excess of pituitary activity is demonstrable after the injections of pituitary extract have ceased, make every clinical investigator aware that similar events might occur in man.

Time Factors in the Cause of Diabetes. The cause or causes of diabetes in man may require variable periods of time to produce the disease. Alloxan damages the islands in a matter of minutes, pituitary extract in four to six weeks. Both the diabetogenic agent and the behavior of the islands will be concerned with the time factor in the cause of diabetes. To complete the picture one need only note that in man obesity is commonly present for twenty years or more before diabetes appears. Certainly no time limit of the causative agent has been set for man.

Vascular Complications. The so-called vascular complications, especially those of the retina and kidney, which are fairly specific lesions of diabetes, may become the subject of laboratory study. Two groups of investigators[36,37] have reported the development of renal lesions in rats within a few months after they had been made diabetic, and Janes and Bounds[38] have demonstrated an abnormality of the retinal capillaries in diabetic rats. If lesions in any way comparable to those which usually take ten to fifteen years to develop in man can be produced in less time and so studied in the laboratory, it should be a forward step in the attack on these complications. Dr. E. H. Rynearson[39] reported that one patient depancreatized for intractable hypoglycemia developed diabetic retinopathy twelve years later. Such information accruing simultaneously from laboratory and clinic should stimulate future investigation.

SUMMARY

A few selected possibilities about the nature, causation and measurement of experimental diabetes have been outlined. A modest comprehension of these experimental experiences should be of some help in the management and study of the disease in man.

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Case Reports

Treatment of Scleroderma, Sclerodactylia and Calcinosis by Chelation (EDTA)*

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THIS is a report of our observations concerning the use of a chelating agent, ethylenediamine tetracetic acid (EDTA), in a patient with scleroderma, sclerodactylia, calcinosis and, possibly, rheumatoid arthritis. Chelating agents have been used medically in cases of lead poisoning;[1] for the removal of urinary calculi;[2] to dissolve calcific opacities in the eye;[3] and to dissolve metastatic calcium deposits in the kidney.[4]

EDTA combines with metal ions to form cyclic complexes which are water-soluble and virtually undissociated.[5] When EDTA is administered rapidly as the sodium salt a rapid lowering of the serum calcium levels results. This is due to the strong affinity of the sodium salt for calcium ions, forming a complex by chelation. When given rapidly in large doses EDTA may cause hypocalcemic tetany. When given slowly homeostatic mechanisms come into play which tend to maintain serum calcium levels by mobilization of calcium from skeletal reserve stores.[6]

Sodium versenate causes an excess of urinary calcium to be excreted. This excess, expressed in per cent of the expected calcium binding power of this agent (1 gm. di-Na-EDTA complexes with 108 mg. calcium), ranges from 63 to 88 per cent of the injected dose.[7]

CASE REPORT

A forty-five year old woman was admitted to the Greenpoint Hospital on February 16, 1954. The patient complained of muscular aches, joint pains and swelling; weakness and inability to walk, stand or use her hands; the presence of hard nodules on her fingers, wrists, elbows and knees; flexion deformities of her fingers, hands, elbows and knees; and a feeling of constriction in her chest. The onset was in 1944 with intermittent pain and swelling of both knees. In spite of treatment by local physicians her illness progressed until 1949 at which time both hands and fingers were involved. It was noted in 1949 that irregular, hard nodules were forming around the fingers and wrists of both hands and at the left elbow. The weakness increased in both hands and by 1951 she was unable to use them.

In 1951 she was given 100 mg. of cortisone daily and the pain and swelling subsided. In November, 1951, after eleven months on this dosage, she manifested psychic symptoms. Medication was stopped and she was admitted to the Kings County Hospital for treatment of cortisone psychosis. She was discharged after six weeks and then was given seven electric shock treatments by a private physician.

Her pains returned thereafter and in September, 1952, she was started on gold therapy. This was given twice a week for six weeks and then discontinued because of lack of improvement. She continued to grow weaker and the pain, swelling and stiffness in the joints increased. In November, 1952, she was admitted to the Long Island College Hospital because of these complaints. Here she received butazolidin,® 300 mg. per day. This relieved her complaints completely but after nine days of butazolidin therapy exfoliative dermatitis, ulcerative stomatitis and mild leukopenia developed. The drug was stopped but the complications did not clear up. While in the hospital a biopsy of skin nodules showed "calcified nodules of subcutis"; a biopsy of skin from the chest wall was reported

* From the Arthritis Service, Greenpoint Hospital, Brooklyn, New York. Presented in part at the annual Meeting of the New York Rheumatism Association in New York City, April 12, 1955. This work was aided by a grant from the New York Chapter of the Arthritis and Rheumatism Foundation.

as follows: "The epidermis is relatively thin but shows a surface layer of keratin. The rete pegs are shortened and show blunted ends. The dermis is fibrotic and shows alteration in the pattern of distribution of collagen bundles. In some regions these form solid hyalinized sheets.

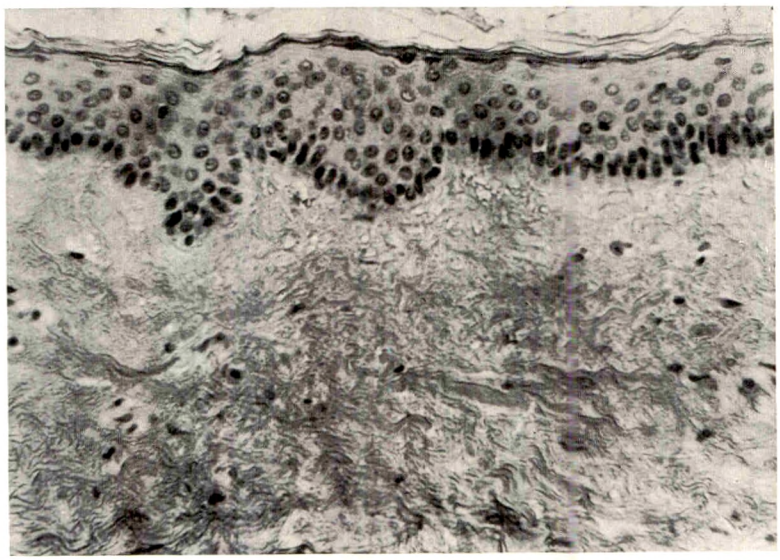


FIG. 1. Skin from right chest wall before treatment. See text for description. (Original magnification × 400.)

The secondary cutaneous appendages are relatively reduced and there are foci of lymphocytic infiltration about capillaries in the dermis. Diagnosis—Scleroderma." (Fig. 1.) The patient was discharged December 16, 1952.

Her skin condition did not improve while at home and she entered the Greenpoint Hospital on January 16, 1953, and was treated by the Dermatology Service. After eight weeks of treatment the skin improved and she was discharged. While at home she took about 50 gr. of aspirin daily for her pain, stiffness and swollen joints.

Family history revealed that the patient's mother had been ill for many years with rheumatoid arthritis.

Physical examination revealed a cooperative, intelligent, anxious female. The patient could not turn or move in bed without assistance. The face was smooth, the features were rigid and without expression. The skin over the face, chest, hands and fingers was "porcelain white," hard, shiny and without elasticity. The finger tips were clubbed and the fingers were held in flexion. (Fig. 2.) There was swelling of the proximal and distal interphalangeal joints. Hard, irregular, nodular masses were present on the fingers of both hands and on the left elbow and both knees. (Fig. 3.) Pressure on these nodules discharged an amorphous, sticky material. Motion in the right wrist was greatly limited in all directions. The left wrist motion was slightly limited due to muscle spasm. Both knees and elbows were swollen, hot and painful. The left knee extended to 130°, flexed to 90°; the right knee extended to 140°, flexed to 90°. Both elbows extended to 140°, flexed to 45°. No hair was present on the fingers, hands, forearms or legs. The chest expansion was from 36½ to 37 inches. The blood pressure was 125/70.

On February 16, 1954, the following tests were performed and the laboratory data were as follows: The urine was negative. The Sulkowitch tests were 1+ to 2+. Blood count: hemoglobin 9.3 gm. per cent, red cell count 3,110 million, white cell count 6.25 thousand. The blood urea nitrogen was 16 mg. per cent; sugar 65 mg. per cent; serum calcium 9 mg. per cent; phosphorus 3.2 mg. per cent; alkaline phosphatase 2.8 Bodansky units. The basal metabolic rate was +40 and on February 27th, +20. The discharge from the finger was found to contain a "moderate amount of calcium." Biopsy of skin from right chest wall showed "partial atrophy of epidermis and appendages. Areas of edema and dense collagenous tissue in the corium with mild chronic focal inflammation." Biopsy of skin and subcutaneous tissue

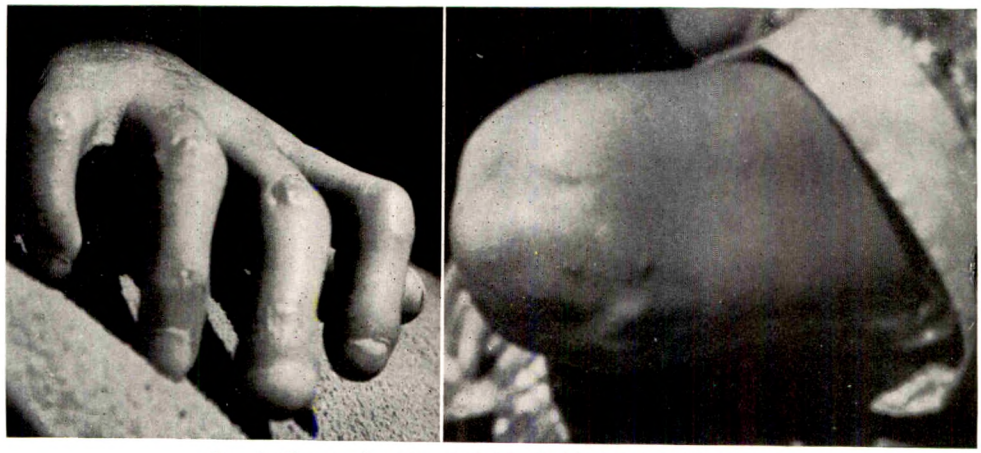


FIG. 2. Fingers flexed and clubbed; skin hard and shining.
FIG. 3. Left elbow; hard, irregular, nodular areas present.

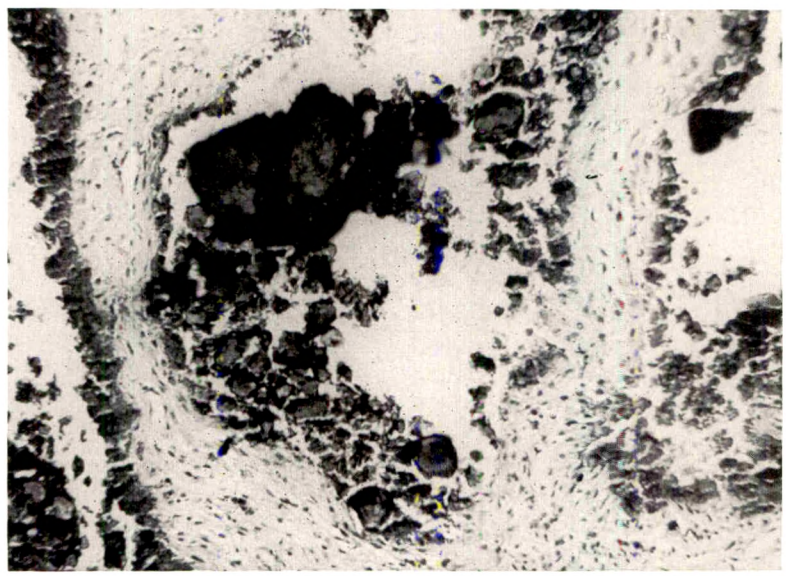


FIG. 4. Skin and subcutaneous tissue from finger before treatment. There is one large and several miniature foci of calcification and slight diffuse and focal chronic inflammation. (Original magnification × 400.)

from the right middle finger showed "one large and several miniature foci of calcification and slight diffuse and focal chronic inflammation." (Fig. 4.)

Blood tests were made on May 4th to check for evidence of rheumatoid arthritis and rheumatic fever. The results were: differential sensitized sheep cell titre = 1:32 (positive); hemolytic streptococcus agglutination, negative; C-reactive protein, negative.

The admission diagnosis was scleroderma, sclerodactylia, calcinosis and possible rheumatoid arthritis.

On May 1, 1954, it was decided to make a trial test of chelation for removal of the calcium deposits. The patient was placed on a diet containing 1.2 gm. of calcium daily. Urinary calcium and blood serum calcium determinations were made. On May 10, 1954, chelation therapy was begun and continued each day for one week. Sodium versenate, supplied by the Riker Drug Co. of Los Angeles, California, was used. This is the sodium salt of ethylene diamine tetra-acetic acid, 20 per cent. (Each 5 cc. of solution at approximate neutrality contains 1.0 gm. in water for injection.) Three grams of



FIG. 5. Scars over proximal interphalangeal joint of left middle finger and right little finger. Shortening of left middle finger.

the drug were dissolved in 500 cc. of 5 per cent dextrose in water. About three and a half hours were needed to complete the intravenous infusion. Twenty-four-hour urine samples were collected and analyzed for calcium content. The calcium determinations were made at two laboratories. At the hospital Mr. Baber used the method described by Hawk, Summerson and Oser.[8] At the Gardner Laboratory Mr. Halpert used the method of Shohl and Pedley.[9] Prior to therapy the patient's urinary calcium excretion averaged 165 mg. per twenty-four hours. During therapy it averaged 293 mg. per twenty-four hours, fluctuating between 210 and 391 mg. The blood serum calcium varied as follows: May 3rd 10.8 mg. per cent; 11th, 7.5 mg. per cent; 12th, 8.3 mg. per cent; 13th, 11.5 mg. per cent; 17th, 9.8 mg. per cent. That part of the calcium bound by the chelating agent was not ashed and could not be determined.

At no time was there any evidence of toxicity, tetany or convulsions. At the beginning of each infusion the patient experienced a mild feeling of heat starting in the arm receiving the medication. This would gradually spread over the entire body and last about two hours after the infusion. The temperature remained normal. There was no flushing or sweating. After the infusion the patient ate and continued her activities. She received physiotherapy, massage and active, passive and resistive exercises regularly. After one week of infusions her chest felt "as if a weight were removed from it." She could breathe more easily. Later she felt "as though all the muscles were loosening up." Chest expansion increased to 37½ inches. Her entire skin felt softer and had lost the porcelain-white look, at times showing a faint pinkish tone. She felt much stronger and moved about in bed with less discomfort than on admission. The aches, pains and joint swelling had decreased. The nodular areas, x-rays of the extremities and flexion deformities were practically unchanged. She was discharged May 22, 1954, improved.

She remained at home until August 25, 1954. During this three-month interval she got around the house in a wheelchair, had pain occasionally, felt stronger and could breathe without difficulty. She noted for the first time that hair was growing on her arms. On July 1, 1954, the proximal interphalangeal joint of the third left finger became red, hot and swollen. The skin broke down, discharging a watery, sticky material. This continued until August 15th at which time two small pieces of bone were discharged. Shortly thereafter drainage stopped and the fistula closed. (Fig. 5.) Aspirin, 40 to 50 gr. daily, kept her comfortable.

She was readmitted on August 25, 1954, to the Arthritis Service, Greenpoint Hospital, because she felt that versene was the first drug that had helped her and desired to give it a longer trial.

Readmission examination was performed on August 25, 1954. The patient's outlook was more cheerful and optimistic. Abduction, left shoulder, to 150°, right to 165°; left elbow

extends to 145°, flexion to 45°; right elbow extension 145°, flexion 45°. The right wrist was immobile, the left was freely movable. There was some wasting of interossei muscles of both hands. The little finger of the right hand was held in flexion. There was clubbing of the right thumb, index and middle fingers. Calcium nodules were felt in the thumb and index fingers. There was clubbing of the left thumb and index fingers. Calcium deposits were present in the left thumb, index, middle and ring fingers. A healed scar was present on the proximal interphalangeal joint of the left middle finger; this finger was ½ inch shorter than on discharge. The function of the hips was good. The left knee extended to 100°, flexed to 90°; the right extended to 130°, flexed to 90°. There were nodules over the left elbow and both knees.

She was placed on a diet containing 1.2 gm. of calcium. After four days of control observations, intravenous medication with the same chelating agent was begun August 30, 1954. This was continued on alternate weeks until October 22, 1954.

Blood chemistry and urine tests were performed and revealed the following laboratory data:

August 25 to October 26, 1954: (1) RBC remained about 3,450,000; hemoglobin, 60 per cent. (2) Urea nitrogen: 17.8 mg. (8/25), 8.3 mg. (9/14), 22.5 mg. (10/27). (3) Bleeding time: 2 min. 10 sec. (9/2), 1 min. 25 sec. (9/13), 5 min. 30 sec. (10/25). (4) Clotting time: 5 min. 45 sec. (9/2), 3 min. 2 sec. (9/13), 6 min. 5 sec. (10/15). (5) Sedimentation rate (Linzenmeier method): varied from 16 min. to 50 min. (6) Total protein: 8.2 gm. (10/5), 9.2 gm. (10/25). Albumin: 2.4 gm. (10/5), 2.7 gm. (10/25). Globulin: 5.8 gm. (10/5), 6.5 gm. (10/25). Inorganic phosphorus was elevated; chlorides were decreased. The other blood findings were normal.

Performance tests were checked and revealed the following information:

September 11, 1954
- Head and neck—Rotation: left, 45°; right, 45° Flexion and extension, good
- Shoulders—Full arm flailing: left, twelve times; right, thirteen times per minute
- Elbows—Supination and pronation: left, all movements good; right, 50 per cent
- Finger tip apposition—Complete
- Fist closure—Right, 30 per cent; left, 0
- Sitting—Flopped into chair and could not rise; could not stand, kneel or squat
- Could not walk, climb, hop or put foot on chair

October 9, 1954
- Head and neck—Rotation, flexion and extension, complete
- Shoulders—Full arm flailing: right and left sixteen times each per minute
- Elbows—Supination and pronation: left, all movements good; right, 50 per cent
- Finger tip apposition—Good
- Fist closure—Right, 40 per cent; left, 20 per cent
- Sitting—Sat without help; did not flop, could rise from chair
- Could stand when wearing shoes; could walk in walker; could not climb, kneel or hop

December 2, 1954
- Shoulders—Full arm flailing: right and left each forty-five times per minute
- Elbows—Supination and pronation: left, all movements good; right, 75 per cent
- Fist closure—Right, 60 per cent; left, 40 per cent

The tests previously cited were repeated during and following treatment. (Tables I and II.)

By September 1st the patient was able to cross her knees for the first time. She felt much stronger. On September 9th the proximal interphalangeal joint of her right fifth finger became red, hot and swollen. The skin broke down, discharging a sticky material. The area healed slowly. (Fig. 5.) On September 10th she began to walk in a walker for the first time in a year. She received codeine and aspirin tablets for pain. By October 7th she felt increasingly stronger and more confident. Her activities of daily living improved steadily. (Table I.) Physiotherapy, exercise and massage were continued. It was noted that a few small nodules had formed on her chin, left elbow, both knees and fingers. By November 15th further subjective and objective improvement was noticeable. A skin consultant on September 21, 1954, found "no evidence of scleroderma." On November 16, 1954, biopsy of skin and subcutaneous tissue from right chest wall showed "The epidermis is not altered. The papillary bodies are better developed than in the section of November 1952, although there are stretches where they are flat

TABLE I

Tests for Activities of Daily Living	Sept. 1, 1954	Nov. 15, 1954
Non-walking Activities		
Bed activities:		
Move from place to place in bed	Poor	Good
Roll to right and then to left side	Poor	Good
Sit erect in bed	0*	Fair
Turn and lie on abdomen	0	Fair
Procure objects from night table	0	Good
Hygiene (toilet activities):		
Comb or brush hair	0	Fair
Brush teeth	0	Good
Shave or put on cosmetics	Fair	Good
Wash hands and face	Fair	Good
Wash extremities	Fair	Good
Manipulate bedpan	0	Good
Adjust clothing for toilet needs	0	Good
Eating activities:		
Cut meat	0	Good
Butter bread	0	Good
Eat with fork	0	Good
Eat with teaspoon, tablespoon	Poor	Good
Drink from glass	Poor	Good
Drink from cup	Poor	Good
Stir coffee, tea, etc.	Poor	Good
Dressing and undressing activities:		
Put on underclothes	0	Fair
Remove underclothes	0	Fair
Put on buttoned shirt (zipper)	0	Fair
Remove buttoned shirt	0	Good
Put on slip-over garment	0	Good
Hand activities:		
Write name and address	0	Fair
Fold letter, place in envelope and seal envelope	0	Fair
Open envelope, remove letter	0	Fair
Use dial telephone	Poor	Good
Turn pages of book	Poor	Good
Wind wrist watch	0	Good
Open and close icebox door	0	Good
Open and close doorlock with key	0	Good
Open and close drawers	Poor	Good
Open and close door hooks	Poor	Good
Pull window shade	Fair	Good
Push door bell	Fair	Good
Use work push buttons	Poor	Good
Work key light switch	Poor	Good
Work pull chair light	Poor	Good
Ring door bell	0	Good
Open and close cabinet lock	0	Good
Turn four-pronged faucet	0	Fair
Turn circular faucet	0	Fair
Open and close medicine chest	0	Fair
Open and close bottle	0	Fair
Open and close safety pin	0	Fair
Strike match	0	Fair
Wheelchair activities:		
Bed to wheelchair	0	Fair
Wheelchair to bed	0	Fair
Propel wheelchair forward 30 feet and stop	Poor	Good
Propel wheelchair backward 30 feet and stop	Poor	Good
Wheelchair to chair	0	Fair
Chair to wheelchair	0	Poor
Elevation activities:		
Bed to erect position	0	Fair
Erect position to bed	0	Good
Wheelchair to erect position	0	Fair
Erect position to wheelchair	0	Fair
Chair to erect position	0	Fair
Erect position to chair	0	Good
Erect position to chair at table	0	Fair
Chair at table to erect position	0	Good
Walking Activities		
Progressing activities:		
Walk forward 30 feet in walker	Poor	Good
Walk backward 30 feet in walker	Poor	Good

* Zero (0) = no activity

and blunted. The subepidermal layer of the dermis is not as compact as in the above mentioned section, although there are stretches where hyaline bands are visible but they are narrow. In its entirety the dermis is much looser and the collagen bundles separated. The sweat glands

TABLE II
GRIP TESTS

	Right (mm. Hg)	Left (mm. Hg)
Aug. 25, 1954	46	86
Sept. 9, 1954	70	86
Sept. 23, 1954	50	50
Oct. 28, 1954	120	100

are large, the basement membranes of the individual units are not conspicuous. The capillaries of the upper corium are far more conspicuous compared with the earlier section." (Fig. 6.) At this time the left elbow became red, hot and swollen. The skin broke down and discharged a pasty material which became hard on exposure to air and crumbled on pressure. Small, hard granules were present in the discharge. A smear of the pus was positive for staphylococci. This infection responded to antibiotics and the elbow decreased in size.

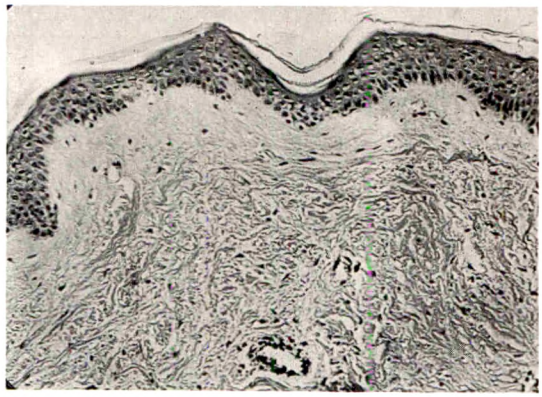


FIG. 6. Skin from right chest wall after treatment. See text for description. (Original magnification × 200.)

Examination on November 19, 1954, revealed a determined, energetic, optimistic individual. She displayed increased agility in getting up in bed, moving on to a chair and then to the walker. The motivation was excellent. The skin of the body and extremities was softer and had increased tone. The face wrinkled when she laughed. The hands and fingers had lost their hard, cold appearance and were softer and wrinkled. Hair was now present on the forearms and legs. Chest expansion was from 36½ inches on expiration to 38 inches on inspiration. The patient was discharged November 20, 1954, very much improved.

On March 18, 1955, follow-up laboratory tests were made to check for possible drug toxicity. The following results were obtained: Sugar, 87 mg. per cent; blood urea nitrogen, 11.9 mg. per cent; sodium, 154 mEq./L.; chlorides, 115 mEq./L.; calcium, 10.3 mg. per cent; and the carbon dioxide combining power, 58 volumes per cent. The total protein was 7.8 gm. per cent (albumin 2.5, globulin 5.3 gm.). Urinalysis: light yellow, clear, acid; specific gravity, 1.004; protein and glucose tests, negative; microscopic examination showed 25 to 30 white blood count and many epithelial cells. The Fishberg test gave results as follows: 8 A.M., 1.011; 9 A.M., 1.006 and 10 A.M., 1.004. The urinary calcium excretion was .067 gm./twenty-four hours. Red blood count was 3.81 million; hemoglobin, 10.8 gm. (70 per cent); color



7 8

FIG. 7. May 19, 1954. Left hand before treatment. Diffuse calcification involving the phalanges.

FIG. 8. January 22, 1955. Left hand after treatment. Decrease in calcification most marked in thumb and middle finger.

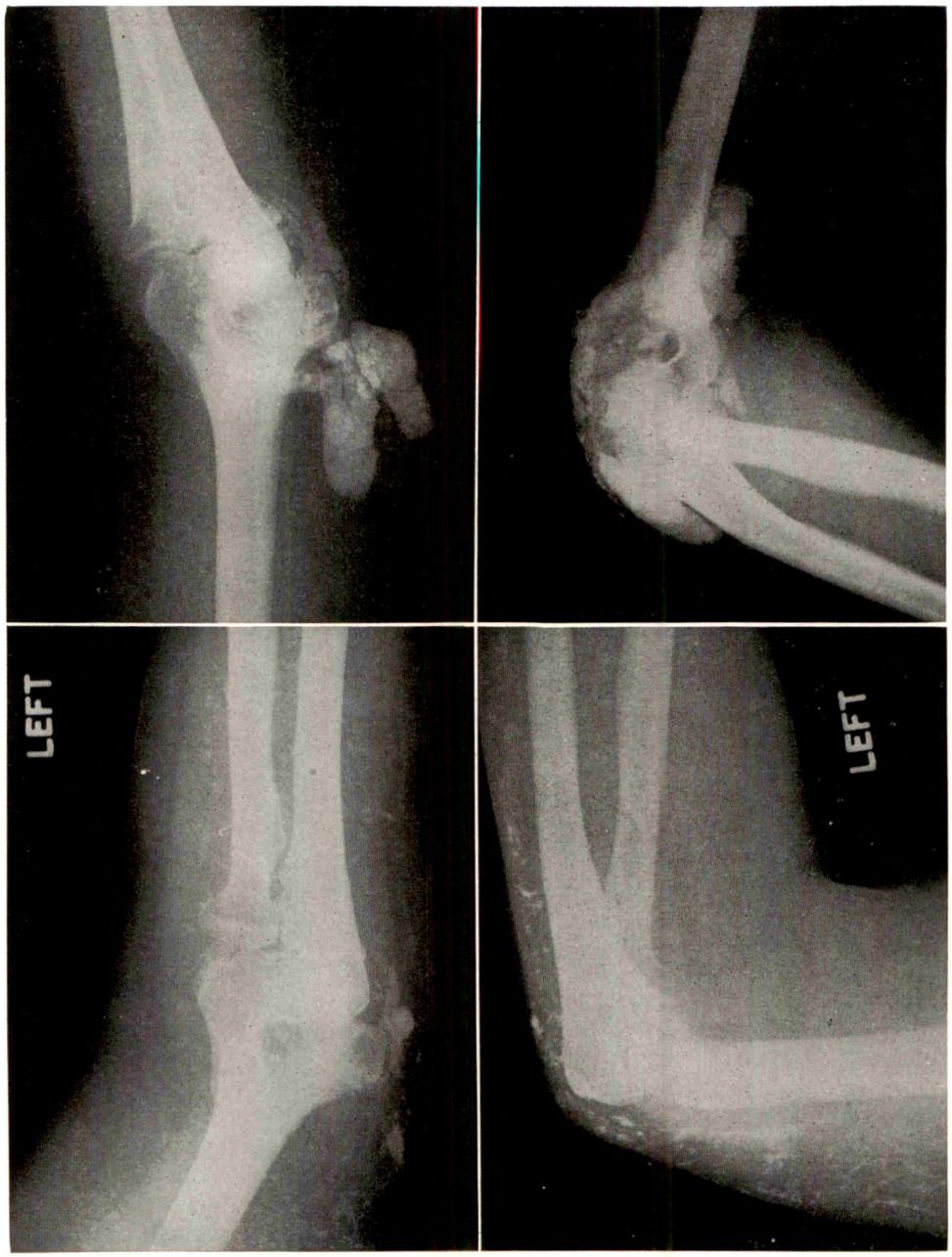


FIG. 9. May 19, 1954. Left elbow before treatment, anteroposterior view. Two large masses of calcium are present.

FIG. 10. August 26, 1954. Left elbow before treatment, lateral view. A large calcific mass present around the joint.

FIG. 11. February 27, 1955. Left elbow after treatment, anteroposterior view. Almost complete disappearance of the calcium deposit seen previously.

FIG. 12. February 27, 1955. Left elbow after treatment, lateral view. Almost complete disappearance of the calcium deposit seen previously.

index, 0.92; white blood count, 7,050; differentials: Schilling cells, segmented, 59 per cent; staff forms, 5 per cent; lymphocytes, 32 per cent; eosinophils, 4 per cent; platelets, 150,000. Prothrombin time was 17.9 seconds and the control was 12 seconds. The first specimen of the urea clearance test was 108 per cent of average normal and the second specimen was 71 per cent of average normal.

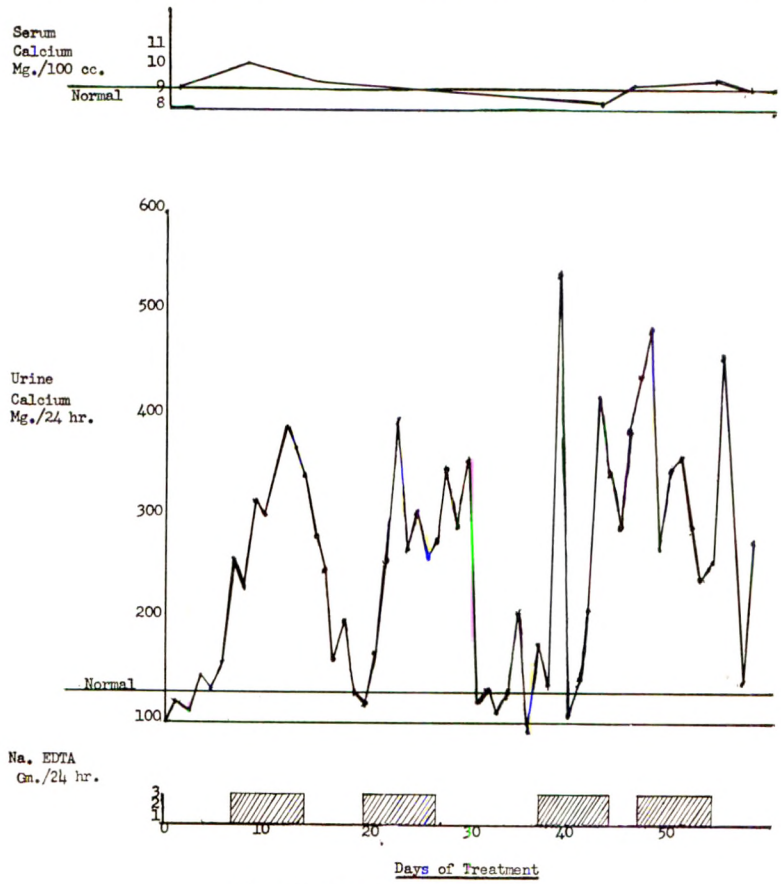


FIG. 13. Response to treatment.

COMMENTS

This patient had a progressive form of arthritis of eleven years' duration. Five years after the onset of her illness she began to show evidence of scleroderma, sclerodactylia and calcinosis. This is a slow, progressive condition with periods of remission and exacerbation. There is no known effective treatment. During her period of observation and treatment in the hospital she was on a fixed diet. This did not affect her calcium deposits.

Since chelation had been used successfully to dissolve urinary calculi, calcific deposits in the eye, and to remove lead from bones, it was thought that it might also remove calcium deposits from the skin and connective tissues.

If the x-rays before and after treatment are compared, marked loss of the calcium deposits is noted. (Figs. 7 to 12.) Table 1 shows distinct improvement in the activities of daily living. This improvement is also shown in her performance tests. Other interesting observations were the softening of the skin and subcutaneous tissues and disappearance of nodules in the left elbow; also, the growth of hair on her forearms and legs. There was a change in the skin biopsy findings. The flexion deformities decreased and joint extension and motion increased.

During treatment there was a definite increase in the output of urinary calcium but no marked change in the level of serum calcium. (Fig. 13.) Bleeding and clotting time decreased; the blood pressure remained unchanged. There was no evidence of toxicity from the drug at any

time during or after medication. An interesting observation is that x-rays first showed evidence of the disappearance of calcium on January 2, 1955, i.e., about four months after the start of intensive therapy.

From the patient's subjective point of view the treatment was of definite benefit. As of March, 1955, the patient felt stronger and had more confidence in herself. She had greater use of her limbs and was able to do many things she could not do previously.

SUMMARY

A patient with typical scleroderma, sclerodactylia, calcinosis and arthritis (rheumatoid?) was treated with a chelating agent, EDTA, given by infusion. Treatment was followed by improvement as indicated by x-ray evidence of marked diminution in the articular and cutaneous metastatic calcific deposits, histologic evidence of regression of the sclerodermatous changes in the skin and return of mobility of the affected joints.

It seems probable that the chelating agent was responsible for remission.

Acknowledgment: The authors wish to express their appreciation to Dr. M. Ziff for his suggestions including the use of the "versenes," and to the Riker Laboratories of Los Angeles, California, for supplies of the sodium versenate.

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Phrenic Paralysis Due to Serum Neuritis

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In routine fluoroscopic examinations of the chest over a period of thirty years, occasional patients have been observed to have paralysis of the diaphragm without satisfactory explanation after careful investigation had excluded the accepted causes of such a finding. In the summer of 1951 the first patient being reported was observed with orthopnea due to phrenic paralysis shortly after a severe episode of tetanus antitoxin serum neuritis. The possibility of this as the source of his trouble was considered, but not being acquainted with any description of phrenic palsy due to serum neuritis, and because he had a vague density in the right upper chest, a tentative diagnosis of Pancoast's tumor was made. Shortly after this the report of Comroe et al.[1] on a similar case appeared and the diagnosis was changed to phrenic paralysis due to serum neuritis. In 1952 another instance of paralysis of the diaphragm was noted and close questioning revealed that the patient had had serum neuritis. A third patient was seen in 1953. He had pneumonia which was slow in resolving. This led to investigation which revealed that he could not cough up his secretions because of weakness of half of his diaphragm. He too had had a severe reaction to tetanus antitoxin three years previously. A fourth patient was found by inquiry among our confrères.

These four patients who had paralysis of the diaphragm have in common only the history of preceding tetanus antitoxin serum neuritis. We believe that these cases merit reporting.

CASE REPORTS

CASE 1. A seventy-one year old white man was seen because of dyspnea on recumbency and pains in his arms. He stated that on July 1, 1951, he had cut his right heel badly enough to require surgical repair and he was given an injection of tetanus antitoxin. Five days later generalized urticaria developed which lasted only forty-eight hours, responding to antihistamine therapy. Following this severe pain developed in both arms, which still persisted at the time of his examination by us on August 22, 1951, some six weeks later. He had noted that the pain was worse in his right arm and shoulder, and that the grip of his right hand was weak. Dyspnea when recumbent, especially after a full meal or when stooping or bending, was noted at about the same time. He had had hypertension for five years, usually averaging around 160 to 170 mm. Hg systolic but occasionally rising to 200 mm. He had not had paroxysmal nocturnal dyspnea.

Examination revealed a well developed, elderly white man who was not cyanotic or dyspneic when erect but quite dyspneic in recumbency. The heart was slightly enlarged to the left, with slight accentuation of the aortic second sound and a faint systolic aortic murmur. The rate was 76 per minute, with regular rhythm. The lungs were clear. The abdomen was normal. The upper extremities revealed no clubbing or limitation of motion. There was slight weakness of the grip in his right hand. There was slight contraction of the palmar fascia in both hands. He had moderate benign prostatic hypertrophy. Fluoroscopic examination showed slight left ventricular hypertrophy and prominence of the aortic knob. The lung fields were clear. The right leaf of the diaphragm was elevated about three interspaces above the left and there were paradoxic excursions, due to phrenic nerve paralysis. A chest x-ray revealed some slight clouding of the right superior sulcus and the elevated right diaphragm. An electrocardiogram was within normal limits. Laboratory studies were not remarkable.

It was thought that he had a superior sulcus tumor with pressure on the right phrenic nerve and so he received x-ray therapy. He was given 1,700 r over the right upper anterior chest between August 28 and September 12, 1951. This produced no change in the patient's symptoms.

A chest x-ray made in January, 1949, was located. This showed the diaphragms were then in normal position. The vague density in the right superior gutter was visible and apparently was vascular in origin.

We continued to think of the possibility of serum neuritis producing phrenic paralysis. When the article by Comroe et al.[1] appeared, the diagnosis was changed in our patient. He has gotten along well since then. When examined on July 3, 1954, he had very few complaints except for prostatism. He has very little dyspnea now, even on recumbency, although fluoroscopy and chest films made on inspiration and expiration (Fig. 1) showed that paralysis of the right leaf of the diaphragm persists. His blood pressure was 210/100.

CASE II. A thirty-seven year old white man was first seen on July 15, 1952, because of pain in the right flank and a story of having passed bloody urine six weeks previously. This was found to be due to a calculus in the right ureter which was removed at cystoscopy by a urologist.

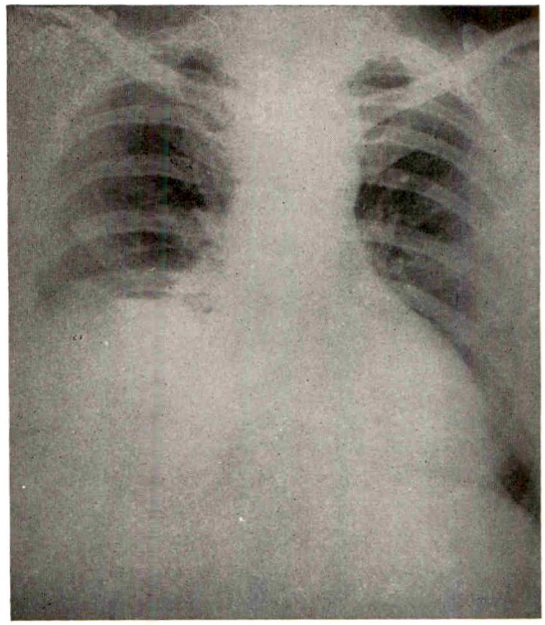
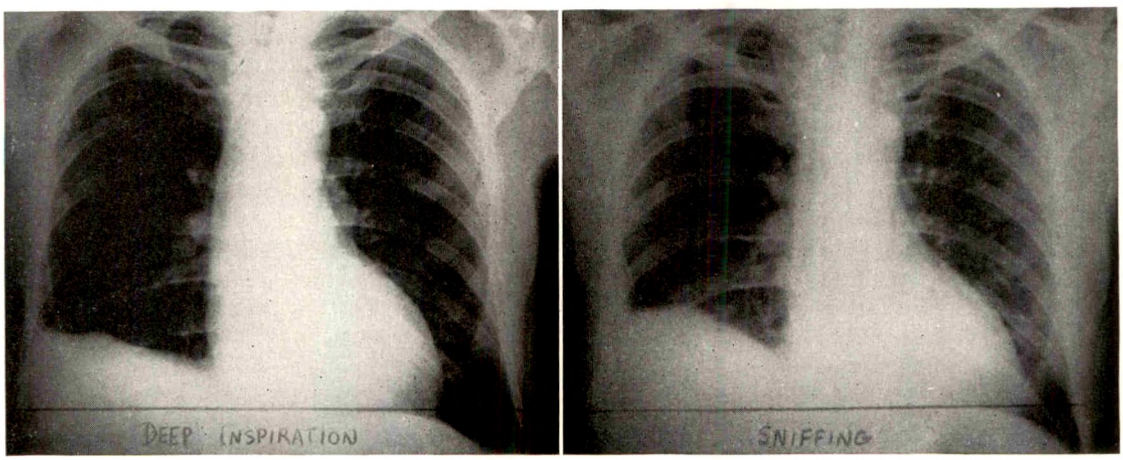


FIG. 1A. Patient No. 1 (August 22, 1951) showing paralysis of the right half of the diaphragm.



1B 1C

FIG. 1. B, July 3, 1954, deep inspiration. C, July 3, 1954, sniffing. The right half of the diaphragm ascends while the left side descends.

He also gave the story that in April, 1951, his truck had overturned. He was bruised but not otherwise injured. He was given an injection of tetanus antitoxin. Within a few hours he had urticaria around the waist and over the rest of his body. Four or five days later he had severe pains through the shoulders and was quite frightened. He felt as if he were becoming paralyzed. He remained home for a few days and then returned to work. He did not recall any definite weakness in the arms or shoulders following the accident or injection of tetanus antitoxin.

Physical examination revealed a well developed, healthy looking young man with no physical abnormalities except for partial paralysis of the left hemidiaphragm noted fluoroscopically and on the chest film. (Fig. 2.) The patient has been seen frequently since 1952 and was last seen January 16, 1954, at which time he was still in good health. The left diaphragm was still almost completely paralyzed while the right diaphragm moved well. There seemed to be a little compensatory emphysema of the right lung.

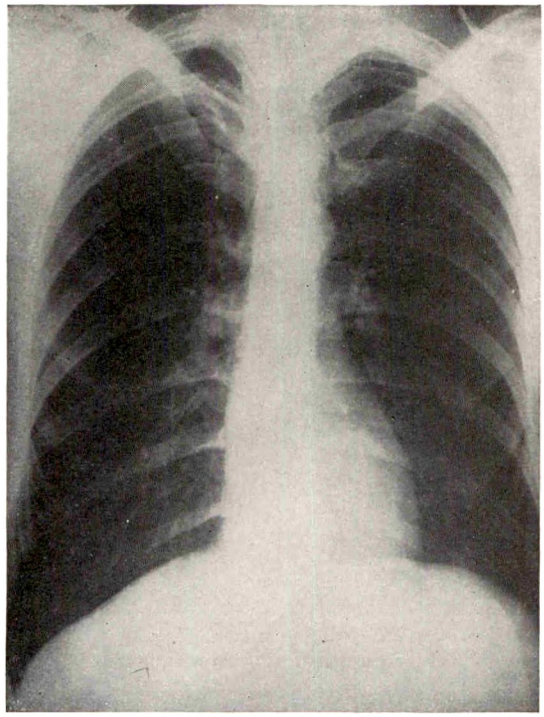


FIG. 2. Patient No. 2 (August 15, 1952). The left half of the diaphragm is paralyzed.

CASE III. A sixty-three year old white fireman was admitted to the hospital in coma on August 29, 1953. He had been in good health until ten days previously when he developed a cold which gradually became worse. The day of hospitalization fever and a chill developed and he soon lapsed into unconsciousness. Examination revealed an obese, comatose, critically ill man who was breathing with Cheyne-Stokes respirations. He was cyanotic. His pupils were pinpoint in size and did not react to light. The neck was supple. All peripheral tendon reflexes were absent. The lungs were full of rales and rhonchi. The heart sounds were almost inaudible, but the rhythm was regular and there were no murmurs or friction rubs. His blood pressure was 95/60. The abdomen was very obese with no palpable abnormalities. There was an old cholecystectomy scar. The optic fundi were normal after dilating the pupils. Lumbar puncture revealed clear fluid with normal pressure; it was reported to contain five cells, all lymphocytes, and 55 mg. per cent protein. X-ray of the chest revealed a small patch of pneumonitis at the right base. Urinalysis was normal. His red blood cell count was 4,660,000 per cu. ml. Hemoglobin was 14.8 gm. per cent. White cell count was 8,450

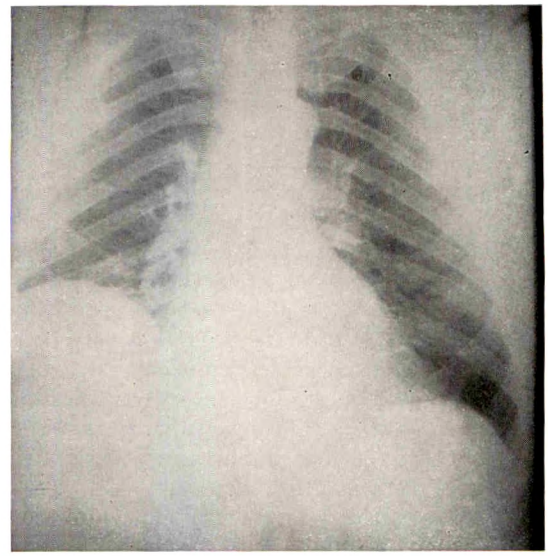


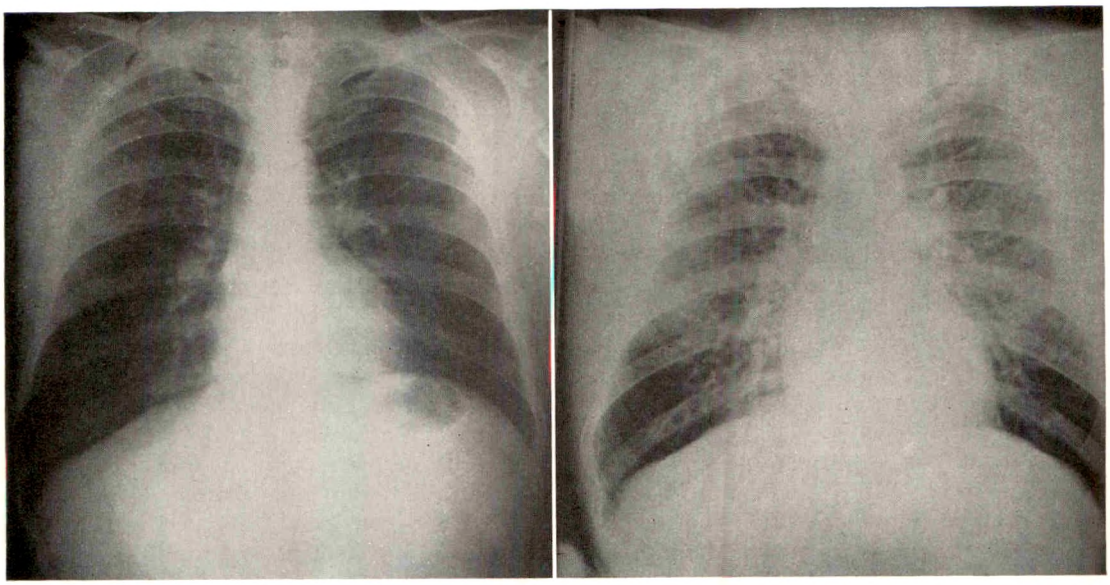
FIG. 3. Patient No. 3 (October 1, 1953). Paralysis of the right leaf of the diaphragm.

with 79 per cent segmented polymorphonuclear leukocytes, 5 per cent stab forms, one juvenile form, 2 per cent eosinophils and 13 per cent lymphocytes. His blood sugar was 158 mg. per cent, and the non-protein nitrogen was 83 mg. per cent. The carbon dioxide combining power was 48 volumes per cent.

The head of his bed was raised quite high and nasal oxygen was started. Within a few minutes he showed striking improvement with clearing of his sensorium and disappearance of the cyanosis. He was then treated with terramycin and adrenal cortical extract. He continued to improve rapidly and looked quite well the next day except for persistent cough. He had many rales in the right chest. His blood pressure was 138/70. He recovered smoothly and was discharged from the hospital in seven days.

Our attention was called to the diaphragm by the first chest film which revealed that the right diaphragm was several inches higher than normally expected. Further questioning revealed that he had had a reaction to tetanus antitoxin three years previously, with arthralgia and urticaria severe enough to require hospitalization. The patient remembered having a good bit of pain in the right hypochondrium and substernal area at the time but he did not remember any peripheral neuritis and we could find no written record of the incident. He had never had a previous chest x-ray.

At our request he came to our office October 1, 1953. He was getting along quite well. Fluoros-



4A 4B

FIG. 4. A, patient No. 4 (May 30, 1949), paralysis of the left leaf of the diaphragm. B, same patient (May 19, 1954). The left diaphragm descends a little more than in 1949.

copy revealed clear lung fields. Both diaphragms descended well on slow, steady inspiration, but on sniffing there was a clear-cut paradoxic motion with the right diaphragm ascending sharply while the left one descended. Films were made in inspiration and expiration. (Fig. 3.) On inspiration the left diaphragm descended 46 mm. while the right side descended only 6 mm. We believed that the evidence indicated definite weakness of the right side of the diaphragm and that it was probably due to injury to the right phrenic nerve as a result of his tetanus antitoxin serum neuritis.

CASE IV. A thirty-five year old white man had an injury to his left hand for which he received tetanus antitoxin on March 30 or 31, 1949. Three days later he noted swelling of the deltoid area of the left arm with redness and tenderness. On April 5th he awakened at 2 A.M. with severe pains in the arms. This soon spread over his shoulders and into the muscles of the lower part of his neck. It later spread to the upper part of his chest bilaterally. He lost the use of his arms to a considerable extent. The pain was intractable and not controlled readily by analgesic drugs. He gradually improved. Physical examination on May 30, 1949, revealed a little external strabismus of the left eye, diminished knee jerks, hypesthesia in both upper extremities, flexion contractures of the right hand, plus considerable limitation of usage of his arms and atrophy of the shoulder muscles, particularly of the right side. The accessory muscles of respiration were thought to be unaffected.

His family doctor remembers quite vividly that extreme difficulty in breathing was the patient's main complaint at the onset of his illness. Fortunately, a chest x-ray was made May 30, 1949, and this film revealed that the left diaphragm was higher than the right, contrary to the usual position. (Fig. 4.) This finding is compatible with weakness of the left side of the diaphragm due to phrenic nerve paresis.

Apparently most of the patient's neurologic defects have improved considerably since then. Physical examination on May 19, 1954, revealed that he still had some weakness and pain in his arms and shoulders. He had a minor reduction in sensation to cotton and pin prick in the right arm. The knee jerks were absent on both sides even with reinforcement. Fluoroscopically the leaf of the left diaphragm was now a little lower on inspiration than the right side. A film showed some weakness of the left side of the diaphragm, although there had been some return of function.

DISCUSSION

There is no way to prove beyond question that the paralysis of half of the diaphragm in each of these patients was due to tetanus anti-

toxin. However, we believe that the circumstantial evidence is strong.

We have listed four cases of persons in various age groups and with widely differing stories. The only points of similarity are the histories of receiving tetanus antitoxin followed by serum neuritis, and the later finding of diaphragmatic paralysis. In addition, the first three had typical urticaria and serum sickness preceding the serum neuritis. Only one of these patients had any clinically detectable residual paralysis of any peripheral nerves. The fourth patient still has some weakness and pain in his arms and shoulders, hypesthesia in the right arm and absent knee jerks.

Involvement of the phrenic nerve might be expected to occur rather frequently in serum neuritis complicating tetanus antitoxin therapy. For some reason this illness affects the fifth and sixth cervical segments preponderantly, usually producing the Erb-Duchenne type of paralysis. The phrenic nerve originates primarily from the fourth cervical segment, but receives a portion of its innervation from the third and fifth segments as well, so it could readily be involved. However, phrenic paralysis due to serum neuritis is not described in the standard textbooks of medicine or neurology, nor is it mentioned in the better summaries of the entire problem.[2–5] We believe that it has frequently been overlooked in the past when routine fluoroscopic examinations of the chest were uncommon.

Respiratory difficulty complicating serum neuritis has been reported infrequently. Dyspnea was listed as a complication of tetanus antiserum used in a twenty-three year old man by Lhermitte.[6] The only specific reference of this nature which we could find in the American literature was reported rather recently by Comroe et al.[1] Their patient had paralysis of the accessory muscles of respiration and weakness of both halves of his diaphragm as visualized fluoroscopically. He began to recover at six months and recovery was practically complete in two years.

Apparently the first report of phrenic nerve paralysis as a complication of serum neuritis was published in the German literature in 1927.[7] A very interesting case was reported by Ladstatter[8] in 1952. He reported a thirty-eight year old healthy man in whom tetanus developed as a result of an injury. Following the onset of lockjaw he was treated with large doses of tetanus antitoxin serum plus penicillin and avertin.[8] He was critically ill. On the eleventh hospital day serum sickness developed. Two days later his lockjaw disappeared and he began to improve. Then right lower lobar pneumonia developed, which cleared. Nineteen days after admission bilateral peroneal palsy was noted. Twenty-seven days after admission a chest x-ray revealed right phrenic paralysis with elevation of the right half of the diaphragm. Another film thirteen days later revealed that the phrenic paralysis had cleared. He was discharged from the hospital approximately three months after his illness began. When last seen, seven months after the onset of his illness, he still had weakness of the right arm with disturbances in sensation and some peroneal palsy. He was given a 50 per cent disability rating at this time.

We are convinced that our patients had tetanus serum neuritis with resulting paralysis of the diaphragm. Our first patient had a chest x-ray made two years prior to his tetanus reaction. The diaphragm appeared normal. When examined six weeks after the onset of the serum neuritis, the paralysis of his diaphragm was present and has remained unchanged ever since.

The second patient has had a persistent paralysis of the diaphragm for two years. He is in excellent general health otherwise. There is no other explanation except as a complication of his serum neuritis.

The third patient had pneumonia. He nearly died because his obese abdomen displaced his weakened diaphragm so high that it led to anoxia, particularly when his ventilation was compromised by the pneumonic process. When he was propped upright in the hospital bed, he promptly awakened as the weight of his stomach fell down from the diaphragm and permitted adequate air exchange. There was no other cause for the paresis of his diaphragm except the story of serum sickness and probable serum neuritis.

The fourth patient was found to have paralysis of the left leaf of his diaphragm fifty-five days after the onset of serum neuritis. His dyspnea appeared simultaneously with the serum neuritis. The paralysis of his diaphragm has improved but is still demonstrable in recent chest films.

Paralysis of the diaphragm is uncommon in otherwise healthy people; and when it is noted fluoroscopically, one is put on the alert for more serious illnesses, primarily neoplasms or injuries along the course of the phrenic nerve. A history

of onset at the time of tetanus serum neuritis could prove helpful in making one feel more complacent as to the cause of the paralysis and as to the prognosis. In our series of four patients, two have no apparent improvement at the end of two and three years, while the other two have shown partial recovery at the end of three and five years. Thus it appears that healing may return after a very long time and we should not be too pessimistic about the long-range outlook of these patients. Fortunately, most of them adjust to the paralysis and have no dyspnea on routine activity long before the nerve function has returned visibly.

SUMMARY

Four cases of paralysis of half of the diaphragm as a complication of tetanus antitoxin serum neuritis are reported. They are all in adult males.

Two patients have shown considerable improvement over a period of years, while the other two have failed to demonstrate any improvement at the end of two and three years.

Routine fluoroscopy of the chest should reveal more of these cases if the possibility of paralysis of the diaphragm due to this cause is kept in mind.

Acknowledgment: Case IV was reported through the courtesy of Drs. George Wilkinson and S. D. Campbell.

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Herpetic Meningo-encephalitis Accompanying Cutaneous Herpes Simplex*

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MENINGO-ENCEPHALITIS is perhaps the least common of the variety of clinical manifestations resulting from infection with herpes simplex virus.[1] In some instances of this disease involvement of the nervous system only is evident. In others a preceding or accompanying herpetic infection of the skin or mucous membranes constitutes the source from which invasion of the central nervous system proceeds. The general impression that it is nearly always fatal is erroneous. Recent serologic studies[2,3] indicate that an increase in antibody to herpes simplex virus can be demonstrated during the course of some apparently benign cases of "aseptic meningitis." Convincing confirmation for the causative role of herpes simplex by isolation of the virus from the spinal fluid combined with serologic evidence has been achieved in only a few cases.

This report will present the clinical and laboratory observations which were made in a patient with herpetic infection in whom meningo-encephalitis was a prominent feature. The study was continued during convalescence and at various intervals for a year after onset of the disease. A considerable amount of interesting and instructive information was obtained regarding the behavior of the virus and the immune response of the patient during and following the episode in which the central nervous system was invaded. The experience which was gained may prove to be helpful in anticipating the problems and difficulties of such study when other cases of this type are encountered.

CASE REPORT

Present Illness. W. G., a thirty-six year old white newspaper reporter, was admitted to the Veterans Administration Hospital in New Orleans, Louisiana, on September 26, 1951. His immediate difficulty had begun four days previously as a sharp constant pain in the lower lumbar region radiating over both buttocks, the posterior aspects of the thighs and the calves, to his heels. This persisted for two days. On the day following the onset of "sciatica" he began to have fever from 99° to 102°F., frequent chilly sensations without frank chills, severe frontal headache spreading over the vertex, soreness of the neck, aching and tenderness of the eyes, and photophobia. He lost his appetite and experienced frequent bouts of nausea. In the evening a fever of 102°F. accompanied by drenching sweat occurred, after which the headache and fever moderated for a short time. He never became irrational or lethargic but complained of difficulty in concentrating his thoughts because of the severe headache.

This episode had been preceded by the development of two peculiar skin lesions which had begun five weeks previously. The first was noted as a small abrasion of the penile foreskin. Another papular lesion, which developed two days later on the left side of the face, was attributed to a mosquito bite. Within a week both lesions had become vesicular and purulent. Treatment with zinc oxide and 5 per cent sulfathiazole ointment did not interrupt their progression and considerable extension. Two weeks after the onset of the skin lesions a physician was consulted. Scrapings from the penile lesion revealed neither spirochetes nor Hemophilus ducreyi. A serologic test for syphilis was negative. After observation for ten days he was given 2 gm. of terramycin daily for four days without appreciable benefit. The symptoms which were immediately responsible for his hospitalization began three days after cessation of the terramycin therapy.

Past History. The outstanding feature in the patient's past history was the discovery of several

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suspicious areas of increased density in both lung fields in the routine chest film at the time of his discharge from the Navy in 1945. He was therefore hospitalized for a two-month period, during which careful clinical and laboratory studies yielded no definite diagnosis. The tuberculin test was negative. He was considered as probably having Boeck's sarcoid. Periodic chest films subsequently revealed increasing fibrotic infiltration bilaterally, most marked in the subapical regions. In 1949 he was hospitalized here because of a spontaneous pneumothorax on the right. At this time the chest x-ray, moderate elevation of serum globulin and a negative tuberculin reaction again suggested the diagnosis of Boeck's sarcoid. Since no superficial lymph nodes were enlarged, a biopsy to substantiate the diagnosis was not done.

A special point was made to ascertain whether or not the patient had had "fever blisters" or other lesions compatible with herpetic infection before the onset of the present illness. No such history could be obtained.

Physical Examination. The patient seemed to be acutely ill. He complained of severe headache. The temperature was 100.2°F., pulse 100, respiration 22 and blood pressure 118/70. The neck was not stiff. The pharynx was diffusely red. Moderate tenderness could be elicited on palpation of the left upper abdominal quadrant. On the left lateral aspect of the foreskin a red, edematous area measuring 3 by 1 cm. was present, the surface of which consisted of multiple small shallow ulcers. No vesicles, exudates or crusts were present at this time. Slightly above and anterior to the angle of the left mandible there was a 2 by 2 cm. area of skin which was crusted over, slightly purulent and apparently secondarily infected. Immediately above this area two small vesicles containing clear fluid were noted.

Neurologic examination revealed absence of the left ankle jerk. The right ankle jerk was hyperactive, with poorly sustained clonus. Babinski's sign was absent. No sensory abnormalities were noted. The retinas appeared to be normal.

Laboratory Studies. The initial lumbar puncture revealed a pressure of 112 mm. H_2O, and yielded clear spinal fluid containing 15 neutrophils and 195 lymphocytes per cu. mm. and 110 mg. per cent of protein. Examination of the blood showed 5.2 million red cells and 16,150 white cells per cu. mm., with 80 per cent polymorphonuclears, 18 per cent lymphocytes and 2 per cent eosinophils. Urinalysis revealed no abnormalities.

No bacteria or fungi were recovered from blood and spinal fluid cultures obtained on admission. Febrile agglutinations provided no significant information. Serologic tests for syphilis were negative. Initial cultures from the facial lesions yielded Staphylococcus aureus hemolyticus only. Neither H. ducreyi nor spirochetes could be demonstrated in scrapings from the penile lesion.

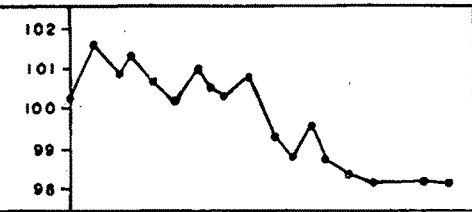
Isolation of Herpes Simplex Virus during Hospitalization. The recovery of herpes simplex virus from the spinal fluid obtained on September 28th indicated the specific nature of the patient's neurologic symptoms and gave a clue to the possible etiology of the skin lesions. Isolation and identification of the virus was accomplished by October 16th. Herpes simplex virus was subsequently recovered from scrapings obtained from the facial lesion on October 19th and from the genital lesion on October 25th. No virus could be isolated from the spinal fluid or scrapings from the facial lesion obtained on October 25th. The spinal fluid findings, white blood count determinations and virus isolations are presented in Table 1.

Course in the Hospital. During the first four days of hospitalization the patient's temperature fluctuated between 100.2° and 101.6°F. The headache, photophobia, anorexia and nausea gradually subsided. During the next three days the temperature steadily decreased to normal. From October 1st until November 25th when he was discharged there was no recurrence of fever or of the other symptoms mentioned.

The facial and genital lesions persisted without evident change throughout the first three weeks of hospitalization except that the vesicles accompanying the facial lesion disappeared during the first week. From September 28th to October 6th he was treated with 0.5 gm. of terramycin every six hours after an initial dose of 1 gm. From then until October 20th 300,000 units of aqueous procaine penicillin twice daily replaced the terramycin. This was then discontinued and aureomycin-varidase ointment was applied locally. From this time on the lesions gradually disappeared. The foreskin healed completely by November 1st and the face was clear by November 25th.

The absence of the left ankle reflex persisted throughout the patient's hospital stay. The

TABLE I



DAY OF HOSPITALIZATION		1	2	3	4	5	6	7	8	16	22	24	28	30	37	44	51	62	76*	107*	118*	278*
DATE		9/26/52	9/27	9/28	9/29	9/30	10/1	10/2	10/3	10/11	10/17	10/19	10/23	10/25	11/1	11/8	11/15	11/28	12/10	1/10/53	1/21/53	4/30/53
SPINAL FLUID	PRESSURE (mm. H_2O)		112	320					Normal	N.D.	N.D.			Normal	200	190	160	150	170		130	130
	NEUTROPHILS per cu. mm.		15	30					5	0	0			0	0	0	0	0	0		0	0
	LYMPHOCYTES per cu. mm.		195	180					215	200	90			50	40	40	25	5	5		10	0
	PROTEIN Mgm. %		110	96					128	148	179			142	107	81	74	88	85		66	58
BLOOD	W B C	16,150	11,750				10,900						10,500		7900							
	% NEUTROPHILS	80	79				79						92		83							
	% LYMPHOCYTES	18	18				12						8		15							
ATTEMPTS TO ISOLATE HERPES SIMPLEX VIRUS	CSF			+										0					0		0	0
	FACIAL LESION											+		0								
	GENITAL LESION													+								
	LESION ON LEFT HEEL																			+		

Explanation for symbols: *represents outpatient visits; N.D., not done; +, herpes simplex virus isolated; 0, no herpes simplex virus isolated.

ankle clonus on the right was not noted again after the first day in the hospital. On October 19th he had double vertical vision for a few minutes and for the next four days noted blurring of vision on rapid turning of the head to the right. This did not occur when he turned his head slowly. Examination of the fundi, determination of the visual fields, and tests of extraocular muscle functions at this time revealed no abnormalities.

During this period of hospitalization further studies were made with reference to Boeck's sarcoid. An x-ray revealed very little change in the areas of fibrosis scattered through both lung fields. A needle biopsy of the liver was performed. Microscopically, granulomatous lesions compatible with those of Boeck's sarcoid were found. Liver function tests were within normal limits except bromsulphalein retention, which initially was found to be 3 per cent but in mid-November was recorded as 12 per cent in forty-five minutes. Repeated serum albumin and globulin determinations were normal. The tuberculin test was again negative.

Subsequent Course. The patient has been seen at irregular intervals after his discharge in order to ascertain the state of his sarcoidosis and to follow the manifestations of the herpetic infection. He returned on January 10, 1952, with a crop of vesicular lesions over the left heel from which herpes simplex virus was isolated. These lesions persisted for approximately two weeks. Since then he has continued to have recurrences of vesicular eruptions every two to three months. Except for one lesion of the right cheek, these have occurred only in the area of the old lesion on the left cheek, and at scattered sites over the lower extremities, most often the left lower extremity. The lesions on the legs usually remain vesicular for a few days and then become crusted over, lasting about three weeks altogether. In contrast, those on the face generally heal completely within four or five days. The recurrent lesions have been assumed to be herpetic on the basis of their appearance. No attempt at virus isolation has been made since January 10, 1952.

In May, 1952, he began to have a rather ill defined aching of the left shoulder and arm occurring every four to six hours. Although readily relieved by aspirin, this indisposition has continued until the present time. Repeated x-rays of the bones have been negative and no cause for the pain has been found.

The left Achilles tendon reflex has continued to be absent. No other neuromuscular abnormalities have developed. Spinal fluid cell counts and protein determinations made since discharge from the hospital have remained within normal limits. Repeated laboratory observations, with particular reference to the sarcoidosis, have shown a slowly rising blood urea nitrogen (present level 29 mg. per cent) and bromsulphalein retention (17 per cent in forty-five minutes). The electrophoretic pattern of a recent serum sample was normal.

VIRUS ISOLATION AND SEROLOGIC STUDIES

Isolation of Herpes Simplex Virus

Spinal Fluid, Sept. 28, 1951. Because of the presence of the genital lesion, lymphogranuloma venereum virus was at first strongly considered as a possible etiologic agent. The initial attempts at virus isolation were directed to this purpose. Spinal fluid in 0.03 cc. amounts was injected intracerebrally into each of four C.F.W. strain mice and in 0.5 cc. amounts into the yolk sac of each of six eight-day old chick embryos.

Of the five embryos surviving longer than twenty-four hours, two died on the sixth day. The presence on the seventh day of pock-like lesions on the chorio-allantois of one of the remaining three was the first indication that herpes simplex virus should be considered. Subsequent microscopic study of this membrane revealed Type A intranuclear inclusions in ectodermal epithelial cells in the focal lesions. A strain of herpes simplex virus was established in the chorio-allantois of eleven- to fourteen-day old embryos. The yolk from one of the embryos dead on the sixth day served as the initial inoculum.

One of the four mice exhibited signs of encephalitis on the tenth day. The other three remained normal and six weeks later were shown to be susceptible to challenge inoculation with the strain of virus derived from the patient's spinal fluid. From the brain of the mouse with encephalitis a strain of herpes virus was isolated and maintained through nine successive intracerebral passages in mice, after which the series was intentionally terminated. Most of the mice in each passage died on the fourth or fifth day after inoculation.

Spinal Fluid Obtained Oct. 25, 1951, December 10, 1951, Jan. 21, 1952, and June 30, 1952. Herpes simplex virus could not be recovered from the

first three samples of this series by inoculation of the chorio-allantois of twelve-day embryos. Three to five blind passages were made before considering each test as negative. The June 30th specimen did not produce signs of encephalitis in six mice during three weeks of observation.

Facial Lesion, October 19, 1951 Swabbings from the base of the lesion suspended in 1 cc. saline containing penicillin and streptomycin produced typical herpetic lesions in the chorio-allantois of twelve-day chick embryos within seventy-two hours after inoculation. Microscopic sections from one of the membranal lesions revealed Type A intranuclear inclusions. A strain of virus was established from this source by passage in the chorio-allantois of eleven- to thirteen-day old embryos.

Facial Lesion, Oct. 25, 1951. Herpes simplex virus was not recovered by inoculation of the chorio-allantois from swabbings collected on this date.

Genital Lesion, Oct. 25, 1951. Vesicle fluid absorbed on a sterile cotton swab and suspended in 1 cc. saline containing penicillin and streptomycin was inoculated on the chorio-allantois of twelve-day embryos. Characteristic membranal lesions appeared in the second passage from this source. Typical intranuclear inclusions were found in sections of the membranal lesions.

Heel, Jan. 10, 1952. Vesicle fluid treated in the same manner as that collected from the genital lesion produced characteristic herpetic lesions on the first passage in the chorio-allantois of twelve-day embryos. Type A intranuclear inclusions were found in sections through the membranal lesions.

Identification of the Virus as Herpes Simplex

A strain of virus derived from each of the four sources in the patient was maintained by passage in the chorio-allantois. Virus neutralization was done by the "pock counting" method, using antiserum prepared by hyperimmunization of chickens with H.F. strain of herpes virus. Previous tests had established a final 1–20 dilution as its effective neutralization titer against several strains of virus obtained from different individuals with herpetic infections. The supernatant fluid obtained by centrifuging a 10 to 20 per cent saline suspension of heavily infected membranes for ten minutes was mixed in equal amounts with a 1–10 dilution of the test serum and incubated at 35°c. for two hours. Virus suspension in an equal amount of sterile saline constituted the control. Each of seven or eight twelve- to fourteen-day embryos was inoculated with 0.2 ml. of the mixtures, evenly spread over the chorio-allantois by means of a tuberculin syringe and needle. Pock counts were made at forty-eight to seventy-two hours following inoculation. The results are presented in Table II.

It is apparent that this method produces considerable variations from one test run to another and also varies markedly from embryo to embryo in each test. Taken as a whole, the neutralization tests offer fairly good evidence for identifying the four strains of virus as herpes simplex. The absence of more complete neutralization can be accounted for by a more recently completed study, employing complement fixation, which indicates that this strain is antigenically quite distinct from the H.F. and other strains obtained from various sources.[4]

Behavior of the Virus in Rabbits. The corneas of each of two rabbits were inoculated with a membranal suspension from an early passage of the virus derived from the spinal fluid. Characteristic herpetic keratitis and conjunctivitis developed in each animal. One was sacrificed at forty-eight hours. Typical intranuclear inclusions were demonstrated in the corneal epithelium. The other animal exhibited signs of encephalitis on the fourth day and died on the seventh day following inoculation. Typical herpetic encephalitis was also produced in two rabbits following corneal inoculation with membrane passage virus derived from the heel lesion.

Studies of the Patient's Antibody Response to Herpes Simplex Virus

Fourteen serum samples were obtained at intervals of varying duration between October 5, 1951, and November, 1952. They were stored in sterile rubber-stoppered tubes at 5°c. until used. Virus neutralization tests were performed with nine of the samples of serum collected between October 5, 1951, and October 27, 1952. Complement fixation tests were run on thirteen samples obtained between October 15, 1951, and November 24, 1952. The strain derived from the patient's spinal fluid was used as test virus in these determinations.

Neutralization Tests on the Patient's Serum. Virus suspensions: The yolk sac of eight-day embryos was inoculated with 0.2 ml. of the supernatant fluid obtained by centrifuging a 10 to 20 per cent suspension of infected membranes from the

fourteenth passage. The amniotic fluid collected from embryos dead on the fourth or fifth day was cultured, pooled after forty-eight hours when found to be free of bacterial contaminants and centrifuged at 2,000 r.p.m. to remove coarse particles. Normal inactivated rabbit serum was added to a final concentration of 10 per cent. This preparation was distributed in equal amounts to glass ampules, flame-sealed, quick frozen and stored at −20°C. until used. Another lot was prepared in the same manner from the fifteenth membranal passage. These are referred to in Table III as Lots A and B, respectively. Each lot was used for about one month after freezing.

Technic of the Test. Phosphate-buffered saline, pH 7.4, containing 10 per cent normal inactivated rabbit serum, constituted the diluent for the virus suspension and the serum in all tests. The test sera, diluted 1:10, were heated at 56°C. for thirty minutes on the day of the test. Preliminary titrations indicated that 0.2 cc. of a final 1–400 dilution of Lot A and the same amount of a final 1–1,000 dilution of Lot B could be expected to produce an average of 100 to 200 pocks on the chorio-allantois of twelve-day embryos. Equal amounts of a 1–200 dilution of Lot A and a 1–500 dilution of lot B and a selected range of fourfold dilutions of the serum samples were mixed and incubated at 35°C. for two hours. A normal serum-virus control mixture was prepared in the same manner with a 1–10 dilution of human serum previously demonstrated to contain no neutralizing antibodies to herpes simplex virus. Each of six twelve-day embryos was inoculated with 0.2 cc. of each serum-virus mixture, spread evenly over the chorio-allantois by means of a tuberculin syringe and needle. Pock counts were made after forty-eight hours' incubation at 35° to 37°C. The results are presented in Table III.

TABLE II

IDENTIFICATION OF THE PATIENT'S VIRUS STRAINS BY NEUTRALIZATION TESTS

Date of test	10/24/51		1/25/52		12/22/51		1/22/52	
Source of virus	Spinal Fluid		Facial Lesion		Penile Lesion		Heel Lesion	
No. of embryo passages prior to neutralization test	Mouse 2 → Embryo 1		4		5		3	
Age of embryos	14-Day		13-Day		14-Day		12-Day	
Approximate pock counts	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum
Embryo No. 1	>200	3	>300	>200	>200	30	>300	>200
No. 2	>300	20	>300	150	>200	15	>300	>300
No. 3	>300	4	>300	200	>200	50	>300	50
No. 4	0	1	>300	100	>200	10	>300	100
No. 5	60	100	>200	200	>200	>200	200	75
No. 6	>300	25	>200	100	>200	>200	>300	250
No. 7	>300	15	>300	>300	>200	25	>300	75
No. 8	Dead	25	.....	.....	>200	>200	.....	.....

It is to be emphasized that the "pock counting" technic provides information only as to relative differences in neutralizing antibody levels. It is not sufficiently exact to provide strict quantitative comparisons between different serum samples.

The results of these tests nevertheless indicate that herpes neutralizing antibody in low concentration was present in the first sample of serum collected during the time the patient had meningitis. The antibody concentration increased slowly during the following five weeks

and then dropped to low levels. It is interesting to note that the titer was found to be low on January 10, 1952, at the time of recurrence of the cutaneous herpes over the heel. The subsequent rise observed by January 21, 1952, apparently represented a response to the recurrence of the infection at that time. It is difficult to interpret the significance of the antibody levels obtained on June 30, 1952, and October 27, 1952, in relation to the frequent recurrences of cutaneous infection experienced by the patient.

TABLE III

TITRATION OF PATIENT'S SERUM SAMPLES FOR HERPES SIMPLEX VIRUS NEUTRALIZING CAPACITY BY THE "POCK COUNTING" TECHNIC

Serum date		10-5-51	10-15-51	10-25-51	11-15-51	12-10-51	1-10-52	1-21-52	6-30-52	10-27-52
Virus lot		A	A	A	A	B	A	A	B	B
Date of test		10-27-52	10-21-52	10-27-52	10-21-52	11-20-52	10-28-52	10-28-52	11-19-52	11-23-52
Final serum dilution	1:20	7/160	22/166	13/160	8/166	6/115	19/151	1/151	0/152	0/97
	1:80	59/160	51/166	31/160	19/166	32/115	37/151	5/151	1/152	1/97
	1:320	86/160	84/166	46/160	33/166	48/115	47/151	36/151	8/152	22/97
	1:1280	.....	.....	86/160	91/166	155/115	.....	72/151	46/152	100/97
	1:5120	.....	.....	.....	.....	.....	.....	.....	117/152	113/97
Serum titer*		1:39	1:34	1:92	1:320	1:49	1:45	1:246	1:735	1:265

Numerator—Average pock count obtained with the serum dilution.
Denominator—Average control pock count.
* Serum titer expressed as the dilution estimated by interpolation to produce 80 per cent reduction in pock count.

Complement Fixation Tests. Thirteen serum samples collected between October 15, 1951, and November 24, 1952, were tested for their capacity to fix complement with herpes simplex antigen. In general, the technic followed was that described by Enders and Levin,[5] employing 0.1 cc. each of antigen and serum and 0.3 cc. of complement. After fixation overnight in the refrigerator at 5°C. the tests were completed by addition of 0.25 cc. of sensitized 1 per cent sheep cells. After incubation for thirty minutes in the 37°C. water bath, tests were read immediately. The highest serum dilution giving 3+ (50 per cent) or greater fixation was then taken as the endpoint.

All dilutions were made in 0.85 per cent NaCl solution containing 100 mg. $MgSO_4$ per L. Pooled serum from four to six guinea pigs, stored in small aliquots at −20°C. and used within five days after collection, constituted the source of complement. A washed 2 per cent sheep cell suspension sensitized with an equal volume of 1:1000 dilution of commercial anti-sheep cell hemolysin was used as the indicator system for all complement titrations and tests. Complement was titrated on each day that tests were set up to determine the dilution which would give two hemolytic units in 0.3 cc. The actual dilution used in the tests varied from 1:53 to 1:100.

The strain of herpes simplex virus derived from the patient's spinal fluid served as the antigen. Considerable difficulty was encountered in obtaining a satisfactory stable antigen suspension with which comparable results could be obtained. Amniotic fluid aspirated from embryos dying three to four days after inoculation into the yolk sac seemed to be best suited to this purpose. Bacteria-free fluids were pooled, centrifuged at 1,500 r.p.m. for fifteen minutes, stored at 5°C. and used within two to three days. The antigen suspension was titrated in serial two-fold dilutions against a 1:4 dilution of each of three positive serum samples obtained from individuals with recurrent herpes. The highest dilution of antigen giving complete fixation with two of the three sera was designated as "1 unit" and twice this amount was then used for the tests. As noted in Table IV, only one antigen suspension was used in a dilution higher than 1:2.

The serum samples were all tested simultaneously, using the same reagents. There was thus considerable variation in the length of storage of different samples. Several samples were found to be anticomplementary. Before each test, aliquots of a 1:4 dilution of each serum were heated at 60°C. for twenty minutes and then reheated at 62°C. for fifteen minutes. Twofold dilutions ranging from 1:4 to 1:128 were set up, using a clean pipette for each dilution. A 0.1 cc. amount of each dilution, delivered to a clean tube, was used for the test.

The following controls were included in each test: (1) Serum controls: (*a*) serum plus normal amniotic fluid in 1:2 dilution; (*b*) serum alone; (*c*) serum plus 2 units of complement; (*d*) serum plus 1 unit of complement. Serum which produced more than 1+ fixation (10 per cent) with 1 unit of complement was considered anticomplementary. No fixation took place in the presence of normal amniotic fluid. The negative human serum control was from the same individual providing the negative control in the neutralization tests. (2) Antigen controls: (*a*) antigen only; (*b*) antigen plus 2 units of complement; (*c*) antigen plus 1 unit of complement. The normal amniotic fluid was set up with the same controls. None of the antigen suspensions or normal amniotic fluids was found to be anticomplementary in the dilutions used. (3) Complement controls: 1 unit and 2 unit complement controls were included to test for complement deterioration during the overnight period in the refrigerator at 5°C.

Table IV presents the results obtained by the complement fixation reaction.

The results of the complement fixation tests demonstrate the difficulties encountered in the application of this method to a series of serum samples collected at intervals over a long period of time. At ordinary refrigerator temperatures serum is likely to become anticomplementary upon prolonged storage. This interferes with attempts to measure the complement fixing titer of the entire group of samples against a single antigen preparation. Considerable variation in results is further encountered when the same serum samples are tested with different antigen preparations at successive intervals. A stable antigen preparation is not yet available which can be used to determine the titer of each serum sample within a short time after its collection. The results here presented permit only the limited conclusion that complement fixing antibodies could be demonstrated in successive serum samples drawn from the patient at intervals between October 15, 1951, and November 24, 1952. There appears to be some fluctuation in complement fixing titer during this period.

TABLE IV

COMPLEMENT FIXATION TITERS OF SERUM SAMPLES COLLECTED FROM OCTOBER 15, 1951, TO NOVEMBER 24, 1952

Date of Test	Antigen Dilution	Date of Serum Sample											
		10-15-51	10-25	11-8	11-15	11-27	12-10	1-10-52	1-21	6-30	8-25	9-29	10-27	11-24
8-21-52	1:8	1:8	1:8	1:8	1:8	1:8	—	<1:4	—	1:64	—	—	—	—
1-9-53	1:2	1:32	1:32	AC	1:64	1:64	AC	1:16	AC	AC	1:64	AC	1:64	1:64
1-19-53	1:2	1:16	1:32	—	1:16	1:16	—	1:4	—	—	1:32	—	1:32	1:32
2-9-53	1:2	1:8	1:16	—	AC	AC	—	1:4	—	—	AC	—	AC	1:32

AC represents anticomplementary; —, not done.

Serologic Tests with Other Viral Agents. Serum samples collected on October 5, October 10, October 25 and November 15, 1951, were sent to the Communicable Disease Center Laboratories in Montgomery, Alabama. No complement fixing antibodies for mumps, lymphogranuloma venereum, eastern and western equine encephalomyelitis or lymphocytic choriomeningitis could be demonstrated in these specimens.

COMMENTS

The specific nature of this patient's illness became evident when lesions compatible with those produced by herpes simplex virus were

observed in embryonated eggs inoculated with spinal fluid obtained during the early stages of the meningo-encephalitis. This observation led to isolation of the virus from the cutaneous lesions. Clinically, the involvement of the central nervous system appeared to be a self-limiting process. The cutaneous eruption can be considered to be the primary source for infection of the central nervous system. The serologic studies, which demonstrate a gradual but definite increase in specific antibody titer, provide further evidence that herpes simplex virus was the cause of the infectious process.

It seems reasonable to believe that this was a first or "primary" infection. No history could be established to indicate the occurrence of previous "fever blisters" or other manifestations of herpetic infection. Since the first serum specimen tested was obtained five weeks after onset of the initial cutaneous lesion, examination of an "acute" specimen was not possible. The demonstration of the absence of antibodies in the acute serum specimen would have provided more satisfactory evidence for the "primary" nature of the process.

Problems regarding the pathogenesis of the central nervous system infection and the recurrent skin eruptions provide interesting speculation in the light of the available data. At the time central nervous system involvement was evident, circulating antibodies could be demonstrated. If invasion by the blood stream accounted for this localization, it apparently took place early in the course of the initial cutaneous eruption. It is unlikely that a viremia would persist for any length of time when specific antibodies, presumably developing in response to the cutaneous infection, had made their appearance in the blood stream. Invasion by propagation along nerve axons from the initial, or genital, lesion would probably be less affected by circulating antibodies. Clinical evidence, particularly the sciatic nerve pain, gives support to the idea that a centripetal spread from the initial lesion along nerve tracts may have constituted an important phase in the pathogenesis of the central nervous system involvement. There is good reason to explain the skin eruption over the left heel as resulting from a centrifugal spread of the virus along nerve tracts. The regular recurrence of cutaneous lesions is perhaps best explained on the basis of persistence of the virus in the central nervous system.

The strain of herpes virus isolated from the spinal fluid of this patient has proved to be of considerable interest. Neutralization tests with anti-H.F. serum suggested divergence in antigenic characteristics. This observation has led to a detailed antigenic analysis by complement fixation of several strains of herpes virus obtained from patients with other manifestations of herpetic infection.[4] It has become evident that this strain is antigenically quite distinct from the majority of the others thus far compared. Whether or not this antigenic difference reflects a difference in behavior of the virus, as indicated by the long duration of the cutaneous lesions, is a matter for speculation. It is interesting to note that the recurrent lesions of the face follow a course more like that of the usual recurrent labial herpes than do those of the lower extremities, which are of a more protracted nature. The pathogenesis of these episodes in relation to the patient's immune response remains an unsolved problem. In what manner, if any, the sarcoidosis may alter this patient's reaction to herpetic infection is not evident. Answers to these questions await satisfactory elucidation of the pathogenesis of the more common types of recurrent herpes simplex.

The experience gained from this case emphasizes the importance of previous familiarity and precedent as a guide in the study of cases of this type. Long-term comparative serologic studies are especially difficult. Quantitative comparisons of the antibody titer by means of complement fixation tests are made difficult by the tendency of serum to become anticomplementary on storage at ordinary refrigerator temperatures. Storage of sera in the frozen state is probably more satisfactory from this standpoint. A stable antigen preparation has not yet been devised. Although neutralizing antibodies are more likely to remain stable on storage, strict quantitative comparisons of neutralizing antibody levels are difficult to achieve. The serologic data obtained from this case indicate that the antibody level against herpes simplex fluctuated considerably. The relation of these fluctuations to the recurrent episodes of cutaneous infection is not clear in every instance. The answer to this and other problems awaits more thorough and consistent study of other cases.

Acknowledgment: We wish to thank Dr. G. John Buddingh for his helpful suggestions in the study of this patient and presentation of the data.

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Fibrinolytic Purpura in Acute Leukemia*

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In a number of different diseases the abnormal occurrence of active fibrinolytic enzymes in the blood may result in severe bleeding.[1–18] Enzymatic dissolution of blood clots with hemorrhage has been described in traumatic shock,[1,2] following thoracic[3,5,6] and abdominal[3,4] surgery, in pathologic pregnancy such as Rh incompatibility[7,8] or abruptio placenta,[9,10] in severe liver disease,[11] in septicemia[12] and as a complication of blood transfusion.[13,14]

The association of fibrinolysis with certain types of malignancy has been noted recently.[15–18] It has been demonstrated[16,19] that prostatic cells secrete a proteolytic enzyme, in effect identical with fibrinolysin, so that an increase in prostatic neoplastic tissue mass or its dissemination throughout the body might result in an increase in the number of functioning cells that produce this substance. Fibrinolysis has neen noted also in association with carcinoma of the pancreas[17,18] but a similar relationship between a fibrinolytic enzyme and the pancreatic secretion has not yet been demonstrated.[17]

Fibrinolysis in leukemia is little known, having been the subject of only a few reports. Janbon et al.[20] described a five year old girl who had leukemia associated with bleeding attributed to thrombocytopenia and to a total absence of fibrinogen. These authors did not report actual lysis of the clot by a fibrinolysin. In June 1954, Giraud and his group[21] reported the case of a forty-five year old man with acute granulocytic leukemia in whom hemorrhage was associated with thrombocytopenia as well as enzymatic dissolution of the fibrin clot. In a study of fifty cases of leukemia of various types, Mene et al.[22] demonstrated increased activity against fibrinogen *in vitro* in the plasma of forty-six patients. Zardi[23] demonstrated a similar fibrinogenolytic phenomenon in ten of eleven cases of Hodgkin's disease. In these experiments, sterile oxalated plasma was incubated for four days and daily determinations of fibrinogen were done. Although a progressive fall in the plasma fibrinogen occurred following incubation, none of these patients showed a bleeding tendency and there was no lysis of the fibrin clot once it was formed. Interference with fibrin formation by a peculiar protein was recently described in a case of multiple myeloma, but true fibrinolysis was not described and the patient apparently did not bleed.[24] No further report of fibrinolysis in leukemia has been made with the exception of passing reference to the possibility of this phenomenon in a review on fibrinolysis.[25]

In this report we wish to describe a patient with leukemia in whom a fibrinolysin was demonstrated shortly before death. Although a more complete study of the properties of the fibrinolytic enzyme could not be carried out, the occurrence of fibrinolysis in leukemia is of such clinical import as to call attention to its presence.

CASE REPORT

Patient J. S. (MCGH, 444412), an eighty-two year old man, was first seen in July 1949 at which time a small rectal polyp was removed. This proved to be an adenocarcinoma of low grade malignancy. No evidence of metastasis was present at the time of surgery. The blood count on this admission was within normal limits. Follow-up studies in the outpatient department failed to disclose any recurrence during the succeeding four years. Within the two months immediately preceding the final admission in November 1953, the patient began experiencing brief, transient syncopic attacks and increasing weakness. No tendency toward hemorrhage was present at this time.

On physical examination the patient was pale but did not seem acutely ill. Petechiae, purpura or bleeding from the mucous membranes was not present. The lymph nodes were not palpable. Gingival hyperplasia was not noted. There was a

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soft systolic murmur at the cardiac apex. The liver and spleen were not enlarged. The prostate was of normal size and consistency.

The hemoglobin was 6.25 gm. per 100 ml., the red cells 2.03 million per cu. mm. and the white cells totalled 3,100 per cu. mm., with segmented polymorphonuclears of 1 per cent, band forms 2 per cent, lymphocytes 19 per cent, metamyelocytes 1 per cent, myelocytes 3 per cent and "blast" forms 75 per cent. There were 73,000 platelets per cu. mm., and the reticulocytes numbered 2 per cent. The bone marrow aspirate showed a preponderance of peroxidase-positive blast forms, some with Auer bodies characteristic of acute granulocytic leukemia.

Urinalysis, serum bilirubin, acid and alkaline phosphatase, total protein, albumin and globulin and non-protein nitrogen were all normal.

Therapy was initiated with two units of red cell mass that raised the hemoglobin to 9 gm. The patient developed intermittent bleeding from the site of bone marrow aspiration. A moderate sized hematoma developed in the ear lobe which had been punctured by a blood lancet. Ecchymoses and hematomas developed in the subcutaneous tissues of the extremities. Five days after admission severe hematemesis and melena suddenly developed; the patient became comatose and died shortly thereafter.

Post-mortem examination showed leukemic infiltration into the spleen, liver and kidneys. During the period immediately preceding death massive hemorrhage into the gastrointestinal tract and the cerebral hemispheres developed. No gross or microscopic evidence for recurrent carcinoma of the bowel was noted. The prostate and pancreas were similarly free of neoplasm.

The coagulation time was performed by a standardized Lee-White method.[26] The bleeding time was determined by the Ivy technic.[27] Clot retraction and clot-lysis time were estimated by frequent periodic observation of the clot obtained in the coagulation time test, incubated at 37°c.

The fibrinolytic activity of the serum of the patient on a saline-washed clot obtained from a normal subject was determined by adding an amount of the patient's serum equal to the quantity of normal serum originally present. The tube was incubated at 37°c. and clot lysis noted by periodic inspection.

Fibrinogen was determined as outlined by Quick[28] in fresh plasma and in plasma incubated at 37°c. for twenty-four hours.

The prothrombin time, total prothrombin, prothrombin consumption time and thromboplastinogen activity time all were performed by methods originated by Quick.[28] The thrombin coagulation time was determined by estimating the coagulation time in seconds following addition of thrombin, 0.1 ml. to plasma 0.2 ml. (Table I.) The test for circulating heparinoids was carried out by adding protamine (0.5 per cent), 0.01 ml. to plasma, prior to determination of the thrombin coagulation time. Circulating anticoagulants were sought by mixing varying quantities of fresh whole blood of the patient with that from a normal control subject and determining the coagulation time. Following coagulation all of the tubes were observed for clot lysis.

TABLE I
TESTS OF HEMOSTATIC FUNCTION
(Coagulation time, 18 minutes; prothrombin time, 16.5 seconds; total prothrombin, 9 seconds)

	Minutes After Formation of Clot		
	15	30	45
Prothrombin consumption time (sec.):			
Tube I	12.5	12	11.5
Tube II	....	11	11
Thromboplastinogen activity time:			
Clotting time (sec.)	12.5	12	11.5

On the second hospital day the bleeding time was four and one-half minutes but hemorrhage from the site of puncture started again several hours later and continued for the remainder of the patient's course. The Lee-White coagulation time was eleven and one-half minutes. Clot retraction began in thirty minutes, but the entire clot was completely liquified within one hour. On the other hand addition of the patient's serum to the washed clot derived from normal blood had no such fibrinolytic effect.

The plasma fibrinogen concentration was determined to be 214 mg. per 100 cc. in freshly drawn blood. (Table II.) Although the fibrinogen concentration of a normal person determined at the same time was 337 mg. per 100 cc., a mixture of equal quantities of both plasma samples had a fibrinogen concentration of 272 mg. per 100 cc.

At the end of twenty-four hours of incubation at 37°c., the fibrinogen concentrations in these three tubes were as follows: patient's plasma, 182; control plasma, 214; and mixture of equal parts of both plasma samples, 219 mg. per 100 cc. In the tubes containing the plasma from the patient, the recalcified plasma clot observed at the thirty minute incubation period required for the performance of this determination was a brittle white flocculate and could be mobilized for sodium hydroxide digestion only by centrifuging. If recalcified plasma from the patient were allowed to stand longer, the clot would disappear entirely.

TABLE II
EFFECT OF INCUBATION UPON PLASMA FIBRINOGEN CONCENTRATION

Time (hr.)	Plasma Fibrinogen Concentration (mg. %)		
	Patient	Patient Control 1:1	Control
0	214	272	337
24	182	219	214

TABLE III
THROMBIN COAGULATION TIME

		Clotting Time	
		Patient (seconds)	Control (seconds)
Plasma	0.1 ml.	....	..
Thrombin 1/5	0.1 ml.	12	9
Plasma	0.1 ml.	....	..
Protamine (0.5%)	0.01 ml.	....	..
Thrombin 1/5	0.1 ml.	9.5	.9

The prothrombin time was 16.5 seconds. Impaired prothrombin consumption (11 seconds) was very likely the result of thrombocytopenia, but impaired thromboplastinogen activity time (11.5 seconds) indicated deficiency in thromboplastic activity of the patient's blood. Thrombin coagulation times are listed in Table III. The prolonged clotting time of twelve seconds when thrombin was added to the patient's plasma indicated the presence of an antithrombic substance. Correction of this defect with protamine suggested that this substance was heparin-like in activity. This substance probably had no appreciable significance as a circulating anticoagulant because coagulation time of a normal person was not prolonged when the blood was mixed in varying proportion with that of the patient. (Table IV.) Although the patient's serum had no fibrinolytic effect on a normal washed clot, clot lysis took place following coagulation of mixtures of whole blood from that of the patient and that of a normal person. The Lee-White coagulation time repeated four days after hospitalization showed an increase to eighteen minutes.

TABLE IV
TEST FOR CIRCULATING ANTICOAGULANT

Patient's blood (ml.)	1.0	0.7	0.5	0.3	...
Control blood (ml.)	....	0.3	0.5	0.7	1.0
Coagulation time (min.)	18	7	7	6	5½
Fibrinolysis	+	+	+	+	...

COMMENT

Although thrombocytopenia played a contributing role, the data herein presented indicate that the chief cause of continued hemorrhage in this patient was destruction of the end product of coagulation, fibrin, by a proteolytic enzyme. The development of clinical events suggests that hemorrhage was a preterminal complication which led directly to death. There is no direct evidence to fix the exact time of onset of fibrinolysis in relation to hemorrhage or the basic disease. Symptoms suggesting leukemia were present for about two months and fibrinolysis was demonstrated at least two days before active hemorrhage had started and five days before death.

It is well known[29] that normal plasma contains an inactive precursor of fibrinolysin called "profibrinolysin." It is the potential function of this substance when activated to remove small blood clots following an inflammatory episode and to institute recanalization of clots in larger intravascular thrombi. The events leading to activation of profibrinolysin are not well understood. It has been postulated[30,31] that the tissues contain a profibrinolysin activator or "kinase" which initiates the changes leading to

clot dissolution in the event of injury, tissue breakdown, shock and other pathologic states.[32] That fibrinolysis may be inhibited by naturally occurring substances was suggested by Giraud et al.[21] and by our observations that a normal clot washed in saline was not dissolved by the serum of the patient. This observation probably represents a mechanism which in normal persons is protection against extensive fibrinolysis.

The possibility that the fibrinolysin in this case might also have fibrinogenolytic properties was suggested by diminished plasma fibrinogen values. (Table II.) Evidence of accelerated fibrinogenolysis, of necessity indirect, has been presented in previous *in vivo*[17] and *in vitro*[22,23] studies. It is our belief that a spurious diminution of plasma fibrinogen level might result from the action of fibrinolysin of the fibrin clot of recalcified plasma during the incubation period required by this examination. Also the drop of fibrinogen values from 214 to 182 mg. per 100 cc. following twenty-four hours' incubation of the patient's plasma was paralleled by a diminution of similar magnitude in the fibrinogen concentration of normal plasma incubated under similar circumstances. Thus our studies do not support the possibility that fibrinogenolysis occurred at least *in vitro*. More concrete evidence would have been derived from a study of the life span of transfused fibrinogen had this been done.

The prolonged prothrombin time in this case suggests that fibrinolysin might have destructive properties directed against prothrombin as well as other coagulation proteins. Stefanini and Gendel[33] have presented a case of carcinoma of the prostate which gave evidence that fibrinolysin was associated with marked shortening of the survival time of "purified" coagulation factors of various types. This point could not be investigated further in our patient due to his early demise. Study of the pathology[34] of the tissues removed at post-mortem examination failed to disclose any morphologic evidence of digestion of tissue proteins, suggesting that this enzyme did not possess a general proteolytic effect.

The paucity of published reports indicates that fibrinolysis is extremely rare in leukemia. In the authors' studies of forty cases of leukemia of various types[35] no other instance of fibrinolysis was demonstrated, nor was there any undue deficiency in the amount of plasma fibrinogen. In these cases of leukemia the level of the plasma fibrinogen was not correlated with the leukocyte count. Conditions accompanied by leukocytosis such as inflammation, on the other hand, are characteristically associated with an increase in the blood fibrinogen value. The studies of Georgeatsos and Quick[36] suggest that products of leukocytic breakdown, injected into experimental animals, are capable of stimulating the production of fibrinogen. Conversely the possibility that leukocytes, lymph nodes or bone marrow might actually manufacture or carry fibrinolytic enzymes[37] should be studied further.

The usual cause of hemorrhage in acute leukemia is thrombocytopenia associated with replacement of megakaryocytes in the bone marrow by leukemic cells. That thrombocytopenia also played a substantial role in bleeding in our case was evidenced by the appearance of spontaneous ecchymoses, gastrointestinal hemorrhage, low platelet count, absence of megakaryocytes and impaired prothrombin consumption.

Finally the presence of a circulating anticoagulant directed against thrombin was suggested by the increased thrombin time determination. The neutralization of this substance by protamine favored its heparinoid nature. Apparently this heparinoid substance was not present in sufficient quantity to cause a marked increase in coagulation time, nor did it prolong coagulation time of normal subjects.

SUMMARY

1. In an eighty-two year old man, acute leukemia was associated with fatal hemorrhagic phenomena. The chief factor in the mechanism of hemorrhage was destruction of the fibrin clot by a fibrinolytic enzyme.

2. The patient also showed thrombocytopenia due to absence of megakaryocytes, increase in prothrombin time, impairment of prothrombin consumption and increase of a heparinoid substance with antithrombic activity.

Acknowledgement: We wish to acknowledge the encouragement and assistance of Dr. Armand J. Quick in whose laboratory the prothrombin, thrombin and anticoagulant studies were made.

Addendum. Since this paper was submitted for publication an account of fibrinolysis in acute granulocytic leukemia was published in the *Annals of Internal Medicine*, 42: 706, 1955 by Cooperberg and Neiman.

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The American Journal of Medicine

VOL. XIX DECEMBER, 1955 No. 6

Editorial

Pathergic Granulomatosis

THE peculiar tissue changes involving particularly the respiratory tract, kidneys and spleen which were first described by Klinger[1] in 1931 and later by Wegener[2] in 1936 have been called Wegener's granulomatosis or syndrome. Other terms applied to this syndrome include necrotizing granulomatosis and angiitis, respiratory-renal type of polyarteritis nodosa, atypical periarteritis nodosa, giant cell granuloma, granuloma with periarteritis nodosa, rhinogenous granuloma (Wegener's original term), rheumatic vascular inflammation, pararheumatic disorder, Loeffler's syndrome and hemorrhagic and interstitial pneumonitis with nephritis. Recently, because of the appearance of these lesions in asthmatics and of the histologic characteristics found, Ehrlich and Romanoff[3] and Churg and Strauss[4] have called this entity allergic granulomatosis. It is the consensus of most contemporary observers of this disease that a sensitivity phenomenon is involved but the term allergic granulomatosis appears inappropriate since the disease may occur without asthma, and a less restricted term seems necessary. Fahey et al.[5] have complicated the question of nomenclature still further by attempting to separate cases of allergic granuloma from what they designate Wegener's granulomatosis with a predominance of necrotizing granulomatous lesions in the respiratory tract and the usual absence of clinical stigmata of allergy or tissue eosinophilia. Yet they noted one case which had the features of Wegener's granulomatosis together with a history of allergy and tissue eosinophilia. Furthermore, they admit the close histologic similarity between the cases of allergic granulomatosis and Wegener's granulomatosis. Ahlström, Liedholm and Truedsson[6] divide their cases into severe and mild nasopharyngeal types but believe that they are essentially the same.

It can hardly be doubted that this plethora of terms has added to the confusion regarding this disease. Consequently, the writer began a search through the literature in an attempt to devise a term which would be more satisfactory, particularly from the aspect of pathogenesis. During this search, Rössle's definition[7] of pathergy as the totality of the morbid phenomena which can be produced by a state of altered reactivity was noted. This definition fits nicely the prevailing concept of pathogenesis of this type of granulomatosis so the term pathergic granulomatosis was chosen and is offered as most suitable for the complex disease under discussion.

It is proposed that pathergic granulomatosis be divided into two varieties. The first is disseminated pathergic granulomatosis which may be viewed as the total possible expression of the

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pathergic process, with many organs and tissues involved; the second variety, in which only a single organ or a portion of an organ is involved without progression to the disseminated form, is designated focal pathergic granulomatosis.

In disseminated pathergic granulomatosis,[8] cough, weakness, fever, night sweats and hemoptysis may continue for years or a fatal termination may occur within a month. In some cases asthma may precede the development of granulomatosis. Abnormal respiratory signs are observed unilaterally or bilaterally on physical examination. In the roentgenogram, single circumscribed opacities may be visible; they may be mistaken for tumor or abscess. Subsequently, multiple lesions of the lungs appear as the disease progresses, and these are accompanied by signs of involvement of the kidney such as urinary albumin, casts, red cells and even gross hematuria. Finally, uremia supervenes. In other cases a diffuse, bilateral process in the lungs visible in the roentgenogram as a confluent, miliary type of infiltration is accompanied by involvement of the kidney at the onset of the disease. Other clinical manifestations noted in these cases have included bloody rectal discharge, epistaxis, marked conjunctivitis, photophobia, petechiae of the skin and ulcerations of the skin and mucous membranes. The blood eosinophilia which has been noted in these cases is often evanescent and disappears in the acute or terminal phases of the disease.

Examination of the lungs grossly in cases of disseminated pathergic granulomatosis reveals single or multiple firm, well circumscribed masses in some cases and more diffuse lesions in others. Granulomatous lesions are found microscopically, with marked ulceration and occlusion of the bronchi and bronchioles, fibroblastic proliferation, focal necroses with palisaded cells, numerous vacuolated macrophages, giant multinucleated cells, and inflammation and necrosis of arteries and veins. In surgically removed pulmonary lesions, eosinophils are found but these are usually absent in postmortem tissues in which superimposed diffuse necrosis of the granulomatous lesions is striking. Massive or spotty necrosis of the spleen and thrombotic granulomatous glomerulonephritis, in which granulomata, glomerular thrombi, crescents and angiitis are present, are commonly seen at autopsy. In some but not all cases extensive destruction of the nose and accessory sinuses may be observed. Ulcerations of the oral mucous membranes, trachea, skin, gastrointestinal tract and bladder and focal necroses of the lymph nodes may occur. While lesions similar to those found in periarteritis nodosa have been observed in regions such as the gastrointestinal tract, they may be absent.

Bacteriologic studies of lesions of the lungs and other organs give either negative results or yield bacteria which are secondary invaders.

An example of focal pathergic granulomatosis[9] is found in idiopneumonic pathergic granulomatosis. Hemoptysis, unilateral chest pain, night sweats, shortness of breath, wheezing, coughing and fatigue accompanied by unilateral rhonchi and rales occur in this entity. In the roentgenogram, unilateral fairly well demarcated patches of consolidation may be seen in the lungs. The lesions are composed of granulomatous tissue with extensive collagenization and remnants of elastic layers of blood vessels present. Ulceration and occlusion of the small bronchi and bronchioles, as in the disseminated form, may be seen as well as numerous vacuolated macrophages in the parenchyma peripheral to the involved portion of the bronchial tree.

Another possible example of a more restricted type of involvement is seen in pneumonitis of the cholesterol type.[9] Here non-bacterial mucosal ulceration and obstruction of the smaller branches of the bronchial tree due to a hypersensitivity reaction of the Arthus type is held responsible for the massive accumulations of lipoid-filled phagocytes and the fibrosis in the adjacent parenchyma of the lung. These changes are identical with the pneumonitis found peripheral to obstruction of a bronchus by a tumor. Clinically, pneumonitis of the cholesterol type is marked by cough, chills, fever, sweating, anorexia, fatigue, malaise, loss of weight, elevated temperature, hemoptysis, dyspnea, sharp to dull chest pain and cough productive of sputum. Some of these signs may be caused by secondary infection of the obstructed bronchi and bronchioles. In the roentgenogram the lesions of pneumonitis of the cholesterol type vary. Collapsed lobes, opaque masses, nodulation and cavitation may be seen. Surgical resection of

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[9] FIENBERG, R. Necrotizing granulomatosis and angiitis of the lungs and its relationship to chronic pneumonitis of the cholesterol type. *Am. J. Path.*, 29: 913, 1953.

the involved portions of the lung has proved beneficial.

The question as to whether or not certain cases of so-called eosinophilic granuloma of the lung may be included as focal types of pathergic granulomatosis is difficult to answer. Numerous eosinophils may be found in pulmonary lesions of the disseminated form, thus creating the impression of an eosinophilic granuloma. It should be noted that pulmonary lesions similar to those found in disseminated pathergic granulomatosis accompanied by eosinophilia have been called Loeffler's syndrome by von Meyenburg[10] and Bayley et al.,[11] among others.

Another interesting syndrome which may be interpreted as a focal manifestation of pathergic granulomatosis is Cogan's syndrome[12] in which non-syphilitic interstitial keratitis and bilateral deafness are found. Eosinophilia has been observed in some of these cases. Evidence that these cases may be linked with pathergic granulomatosis may be found in the report by Oliver et al.[13] in which subcutaneous granulomatous nodules and angiitis were detected in a patient with Cogan's syndrome. Certainly the published illustrations of the subcutaneous nodules are reminiscent of pathergic granulomatosis.

In the literature pathergic granulomatosis has often been linked with periarteritis nodosa and has been thought of as a borderline type or as true periarteritis nodosa with added granulomatous lesions. The belief has grown that granulomatous lesions are caused by arterial changes but the absence of granulomatous lesions and of phlebitis in cases of extensive arterial lesions in periarteritis nodosa as well as the absence of arterial changes in some cases of disseminated pathergic granulomatosis has been glossed over. Another interpretation may be offered, however, in which the arterial system would be considered a single organ and limitation of the involvement to the arteries would constitute a form of focal pathergic granulomatosis. Thus the arterial lesions in periarteritis nodosa would not lead *per se* to extravascular granulomatous lesions but, in those cases in which lesions of arteries characteristic of periarteritis are present together with extravascular granulomatous lesions, either might be considered a result of the pathergic process and neither would be dependent on the other for its pathogenesis.

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Clinical Studies

Studies on the Mechanism of Ventricular Activity*

XVI. Activation of the Human Ventricle

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THE classic theory of the mechanism of activation of the mammalian ventricular wall evolved from the pioneer work of Lewis and Rothschild in 1915[1] and subsequent observations made by a number of other investigators. In these studies the mode of depolarization of the myocardial wall from endocardium to epicardium was inferred by the study of the electrical potentials in the cavity of the ventricles on the one hand and on the epicardial surface on the other. Electrocardiographic properties of the intramural layers situated between endocardium and epicardium were not studied until recent years.

Activation of the ventricular wall, according to classic theory which is based on animal experimentation, is accomplished by advance of the activation front, at a constant rate, from endocardium to epicardium. All layers of the myocardium are assumed to contribute in proportion to their thickness to the genesis of electrocardiographic deflections. For instance, the R wave of the depolarization complex is said to begin in the endocardium and increase progressively in amplitude as the activation front approaches the epicardium. The S wave is supposed to decrease in amplitude from endocardium to epicardium. This theory was applied to human electrocardiography and has since served as the basis for interpretation of both normal and pathologic electrocardiographic deflections.

Attempts at direct electrocardiographic studies of the human heart were made as early as 1929 when Barker and his associates obtained records by placing electrodes directly on the surface of a heart exposed following pericardiostomy.[2] In 1942 Nylin and Crafoord[3] obtained direct electrocardiograms in two patients subjected to thoracotomy for pneumonectomy. Groedel and Borchardt[4] studied direct electrocardiography in 100 patients with pneumothorax and other affections of the lungs.

The development of the technic of cardiac catheterization made it possible to record potentials from the human cardiac cavities. Thus Lenègre and Maurice,[5] Hecht,[6] Sodi-Pallares and his co-workers,[7] Kisch et al.,[8] Duchosal and associates,[9] Kert and Hoobler,[10] Kossman and co-workers,[11] Wenger et al.,[12] Levine and his associates,[13–15] and others studied potential variations of right intracardiac and intravascular cavities. More recently, Seligman and his associates,[16] Sodi-Pallares et al.,[17,18] Gibert-Queralto and co-workers,[19–21] and others explored the left cardiac cavities with the use of catheter electrodes. Considerable contribution was made to the understanding of cardiac electrophysiology by these studies. However, the electrical phenomena

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occurring within the myocardial wall remained unexplored; the mode of genesis of electrocardiographic deflections between endocardium and epicardium continued to be unknown.

Our interest in further study of this field was aroused when certain electrocardiographic findings obtained in experimentally produced myocardial infarction in dogs could not be adequately explained by the classic concepts: it was observed that an old infarct confined to the subendocardial myocardium did not alter in any way the records taken directly from the overlying normal epicardium. This was at variance with the prevailing concepts concerning electrocardiographic changes of subendocardial infarction which anticipate a QR type of complex.[22,23] If it is assumed that all layers of the myocardium are equipotent in producing R potentials, then necrosis of the subendocardial layers would be expected to cause the initial segment of the R wave to be replaced by a Q wave. The discrepancy could not be resolved without knowledge concerning what takes place during the process of repolarization in the various myocardial layers individually.

The development of a new approach to the study of ventricular activation by use of needle or "plunge" electrodes in this laboratory, and independently by Scher,[24] Sodi-Pallares[25] and Durrer,[26] opened new vistas to the understanding of electrocardiography. This technic enabled us to register electrocardiographic phenomena at any desired level of the myocardium. Depolarization of the inner layers was shown to produce no R wave. Records obtained from these layers yielded pure QS waves rather than rS waves as had been assumed by classic theories.

These findings afforded a tentative explanation for the previous observation that healed subendocardial infarction in dogs was not associated with electrocardiographic changes. Indeed, necrosis or fibrosis of a portion of the myocardium which does not normally contribute to the genesis of the electrocardiographic deflections could not be expected to alter the electrocardiogram.

The technic of intramural electrocardiographic exploration was considered to be informative and, in fact, indispensable for study of the otherwise inaccessible intramural layers. Since it was noted in several hundred dogs subjected to intramural electrocardiography that the procedure was without hazard, similar studies in human beings were considered feasible and the objective evidence resulting therefrom highly desirable.

A review of the literature concerning intracardiac injection of drugs for the purpose of resuscitating the stopped heart revealed that the procedure of cardiac puncture was without untoward consequences in the hearts revived.[27,28] Accidental puncture wounds of the heart, such as those occasionally encountered in the course of pericardial paracentesis and in stab wounds of the heart, are known to be fatal only if they traumatize an important branch of the coronary arteries, leading to hemopericardium and cardiac tamponade.

Clinically, Ponsdomenech and Beato Nunez in 1951[29,30] suggested a technic of cardiac puncture for direct visualization of the cardiac cavities. They introduced a special trocar forty-five times in thirty patients through a subxiphoid transdiaphragmatic route and found the procedure devoid of significant ill effects. In cardiac surgery, Brock's valvotome or other comparable instruments are thrust through the ventricular wall for division of stenotic pulmonic or aortic valves. Bleeding from the site of the valvotome after it is removed is usually minimal and controllable with fingertip pressure.

The extensive experience accumulated during four years of intramural electrocardiographic studies in dogs led us to regard the procedure as completely safe. Intramural electrocardiography was found to be devoid of hazard not only in normal dogs but also in dogs with the following conditions: acute and old myocardial infarction, experimentally produced bundle branch block, ventricular arrhythmias, experimentally produced arterial hypertension and myocardial failure due to hemorrhagic shock. There were no complications attendant upon cardiac punctures even in dogs dying after prolonged manipulation with production of multiple infarcts. No serious complications occurred in animals subjected to numerous plunges on repeated occasions. Isolated premature ventricular contractions, which were always innocuous, constituted the only ill effect attributable to the puncture with plunge electrodes.

It is to be recalled that the plunge electrode used in this type of study is very small, the size of a 22 to 20 gauge injection needle. The amount of trauma to the myocardium is negligible and the bleeding hazard is practically non-existent

when the coronary arteries are avoided under direct vision.

On the strength of these facts and observations, and since we had no reason to fear that the human heart would be more vulnerable to electrode puncture than the dog's heart, a study of human intramural electrocardiography was undertaken.

MATERIAL AND METHODS

The present report is based on the results of intramural electrocardiography in twelve human subjects. However, prior to initiation of this study two patients who were found to have incurable carcinomas during thoracotomy were subjected to this procedure. This was done primarily to determine the response of the functioning human heart to puncture with our minute plunge electrodes. The electrode was introduced to a depth of 5 mm. in the lateral wall of the heart and then removed. There was no bleeding from the puncture sites and it was noted that under direct vision the coronary arteries could be avoided without difficulty. Continuous, direct electrocardiograms revealed only occasional premature ventricular contractions. Postoperative observation failed to reveal any complications.

Subjects. There were twelve patients in whom thoracotomy was performed for treatment of various conditions: carcinoma of the lung in four; rheumatic heart disease with mitral stenosis in three; carcinoma of the esophagus in one; cardiospasm associated with megaesophagus in one; pulmonary tuberculosis in one; cyanotic congenital heart disease in one and possible constrictive pericarditis in one There were six males and six females, ranging in age from twenty to sixty-two years.

There was no clinical or laboratory evidence of heart disease in seven subjects. In the remaining five there was clinical evidence of organic heart disease (three of mitral stenosis, one case of possible constrictive pericarditis and one case of congenital heart disease). The patients with mitral stenosis and congenital heart disease did not, however, exhibit any evidences of intrinsic myocardial disease. There were no signs of rheumatic activity.

Electrodes. The electrodes used in this study were similar to those described previously.[3] They consisted of straight 2 inch long pieces of 0.020 gauge tempered silver wire sharpened at the recording tip. The insulating material chosen for this study was G.E. glyptal which is a water/oil resisting rubber cement with very high viscosity. The electrode wire was introduced into the cement and pulled out gently so as to secure a uniform, smooth coating on the wire. By virtue of its high viscosity, the insulating substance withdraws from the sharp tip leaving the very end bare and conductive. When dry, glyptal possesses remarkable pliability, allowing the electrode to be bent or manipulated without losing the continuity of its insulating coating. The other end of the electrode was connected to electrocardiographic lead cables by means of an insulated fine copper wire. The electrodes were sterilized in strong zephiran® solution before use.

Each electrode was scrupulously tested before and after use for evidence of "leak" or defective insulation. The recording tip was chlorided prior to use. The electrodes were marked at 2 to 5 mm. intervals so that the distance between the recording tip and the epicardial surface could be determined with ease. Soft cotton-tipped electrodes were used for recording of the surface potentials.

Recording Apparatus. Two sets of recording apparatus were used: the direct-writing Sanborn Viso Cardiette for continuous observation of cardiac rate and rhythm and the Sanborn Twin-Beam electrocardiograph which is a photographic apparatus with a very high frequency response and capable of operation at paper speeds of 25 mm./sec. and 75 mm./sec. By virtue of its high frequency response and high paper speed, it enabled us to analyse the deflections with considerable precision.

The standard limb lead II was recorded on the upper channel of the Twin-Beam and used as time reference. The direct intramural lead was recorded in both the direct writing Viso Cardiette and the lower channel of the Twin-Beam. The indifferent electrode was connected to Wilson's central terminal forming a unipolar lead. Intramural tracings were recorded at an attenuation of 20X so that an input potential of 20 m.v. caused a 10 mm. deflection.

The procedure was performed in the operating room with patients anesthetized for surgical treatment of their basic diseases. It consisted of introducing the electrode into the myocardium and recording potentials at various depths of the myocardial substance as well as from the ventricular cavity and surface. The area of the left ventricle most commonly used for exploration was the free lateral wall mid-way between the apex and the base. It was found that the electrode entering the cavity from this area would avoid both papillary muscles.

In only one case was the chest opened on the right, for removal of carcinoma of the right lung. In this case intramural electrocardiography was carried out in the infundibular area of the right ventricle. In the remaining eleven cases the procedure was performed on the left ventricle.

An attempt was made to enter the cavity of the ventricle first and then, by withdrawing the electrode slowly, record from various levels of the myocardium. This was done primarily to secure orientation concerning the location of the electrode tip. Generally, the cavity tracing could be recognized rather easily as the depolarization complex obtained therefrom exhibited a pure, symmetric QS followed by an isoelectric S-T segment. Furthermore, in the process of advancing the electrode into the myocardial sub-

stance and toward the cavity it was possible to determine the exact moment when the electrode tip was against the endocardium by the perception of an elastic resistance. For analogy and simplicity, the consistency of the myocardium can be likened to that of Swiss cheese and the consistency of the endocardium to that of cellophane wrapping paper around it. After exerting additional pressure for traversing the endocardium a sensation of "give" could be perceived as the tip entered the ventricular cavity.

It is pertinent to mention at this point that the thickness of the ventricular wall of a heart is considerably greater during life than it is after death. The explanation for this is not quite clear; however, it appears that the amount of blood contained within the myocardium and the tone peculiar to living muscle cells account, at least in part, for this finding. It is not surprising, therefore, that in our studies the normal human left ventricular wall was found to be 25 to 30 mm. thick instead of 10 to 15 mm. found in normal hearts after death.

The records were analysed for the following: (1) configuration of the QRS-T from the cavity, intramural layers and epicardial surface; (2) characteristics of the injury current incident upon introduction of the electrode into the myocardium; (3) time of arrival of the activation wave at various levels of the myocardial thickness and, secondarily, speed of transmission of the depolarization wave through the ventricular wall. The time reference used for this purpose was the beginning of the QRS deflection in the simultaneously recorded lead II.

The procedure in general was not difficult; however, it was more complicated than comparable procedures in the dog due to the following facts: first, the greater vigor and amplitude of cardiac contractions in human beings made manipulation and holding in place of the electrode somewhat tedious; second, the 60 cycle alternating current was a persistent annoyance in the operating room: it was difficult to eradicate in spite of the availability of competent professional assistance; third, the electrode-induced injury current was more persistent in man than in dogs. Although the injury current did decrease in amplitude when the electrode was allowed to remain in place for several minutes, this was not always feasible in surgical patients as it would prolong the operation.

LEFT VENTRICLE (ELEVEN CASES)

Configuration of the Depolarization and Repolarization Complexes. Depolarization complexes: The depolarization complexes were identical in nine cases, and were considered to represent normal pattern of depolarization. In the remaining two cases (both of mitral stenosis) certain departures from normal were encountered which will be pointed out.

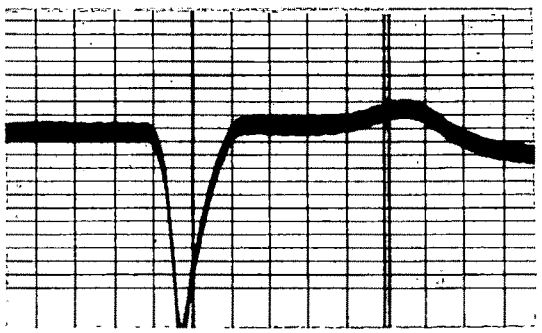


FIG. 1. A typical left ventricular cavity complex with pure QS, isoelectric S-T segment and positive T wave.

The normal cavity depolarization complex consisted of smoothly contoured, pure QS waves with symmetrically ordered descending and ascending limbs. (Fig. 1.) In the two cases of mitral stenosis a notch was present in the descending limb of the QS.

The intramural depolarization complexes differed materially in the subendocardial and subepicardial myocardium consisting in the former of pure QS deflections with no evidence of R wave, and in the latter of rS or qrS. For all practical purposes the myocardial wall seemed to be made up of two zones distinguishable one from the other in the matter of generating R potentials. The first zone, the subendocardial myocardium, was virtually "silent," that is, it did not produce R potentials. Subendocardial QS waves were distinguishable from cavity QS waves by the presence in the former, of small S-T segment elevation ranging in amplitude from 1 to 5 mm. This minimal injury current could be eliminated by allowing the electrode to remain in place for a short time. The second zone, the subepicardial myocardium, was wholly or almost wholly responsible for the genesis of R potentials. The first evidence of R wave usually began at a level almost equidistant from endocardium and epicardium and gradually increased in size as the electrode was moved out towards the epicardial surface. (Fig. 2.) The R/S ratio increased progressively in this zone. On the epicardial surface the depolarization complex consisted of qRs or Rs.

In the two cases of mitral stenosis the notch observed on the descending limb of the cavity QS waves persisted throughout the myocardial thickness.

Repolarization complexes: Cavity and intramural T waves were positive in all cases. Surface T waves, on the other hand, were positive

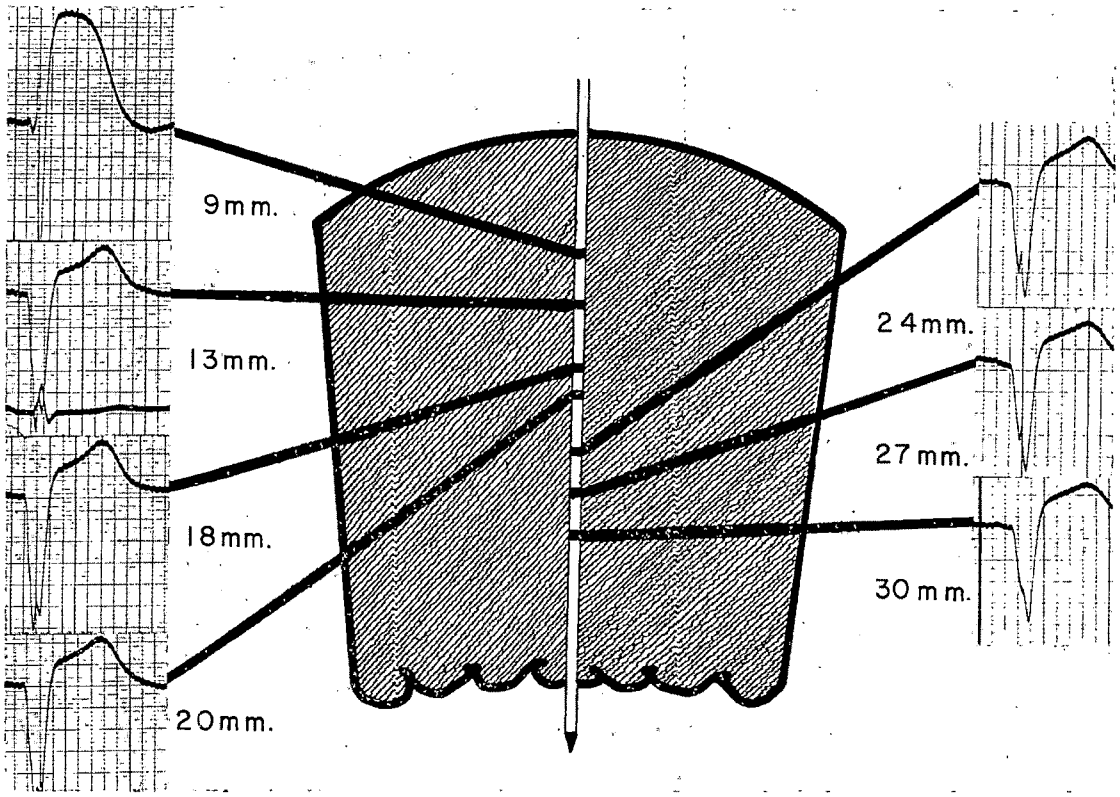


FIG. 2. Records obtained from various depths of the myocardium and from the surface. All the records taken from the inner half of the myocardial thickness are identical in every respect. The depolarization complexes obtained from the inner 15 mm. of the myocardial thickness consist of pure QS waves. At 12 mm. there is a small R wave and an S wave which is smaller than the S wave of the deeper layers. On the surface the complex shows no S wave. The S-T segment is isoelectric in all records taken from the cavity to a distance of 18 mm. from the epicardial surface. S-T segment elevation makes its appearance at 15 mm. below the surface and progressively increases in size as the electrode is moved toward the epicardial surface.

in ten cases (Fig. 3) and flat to slightly inverted in one case. The amplitude of the T waves obtained from cavity, intramural layers and epicardial surface was equal in nine cases. This is considered to be the normal pattern for the T waves. In the remaining two cases, however, there was a gradual change in the amplitude: increase in endoepicardial direction in one and increase in epiendocardial direction in the other. (Fig. 4.)

S-T Segment and the Electrode-induced Injury Current. S-T segments were invariably isoelectric in leads recorded from the cavity. There was slight S-T segment elevation in leads recorded from the inner myocardial layers. As shown in Table I, the electrode-induced injury current of the subendocardial layers ranged in amplitude from 1 to 5 mm. with a mean value of 2.75 mm. and standard deviation of ± 0.46. Under no circumstances was a monophasic curve or even a high injury current obtained from this portion of the myocardium.

The electrode-induced injury current in the superficial myocardial layers was generally high, ranging in amplitude from 15 to 30 mm. with a mean value of 22.66 mm. and standard deviation of ± 2.19. (Table I and Fig. 5.) Monophasic curves were commonly observed in tracings taken from the outer myocardial layers. The injury current in this portion of the myocardium, unlike that of the deep layers, persisted for periods exceeding ten minutes. (Fig. 6.) QRS and T deflections were readily distorted by high injury currents. Consequently, complexes recorded from the superficial myocardial layers which retained their injury current did not always yield to accurate interpretation.

Epicardial surface complexes were obtained by the use of cotton-tipped soft electrodes, hence no S-T segment elevation was observed. How-

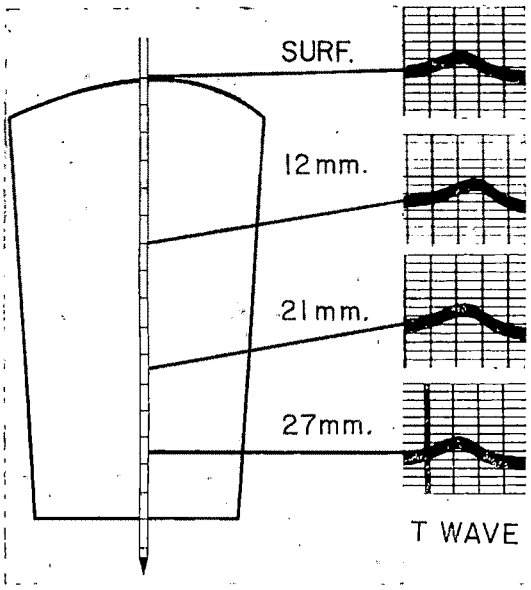


FIG. 3. Intramural and surface T waves obtained from a normal left ventricle. Note that all T waves are identical in direction and amplitude.

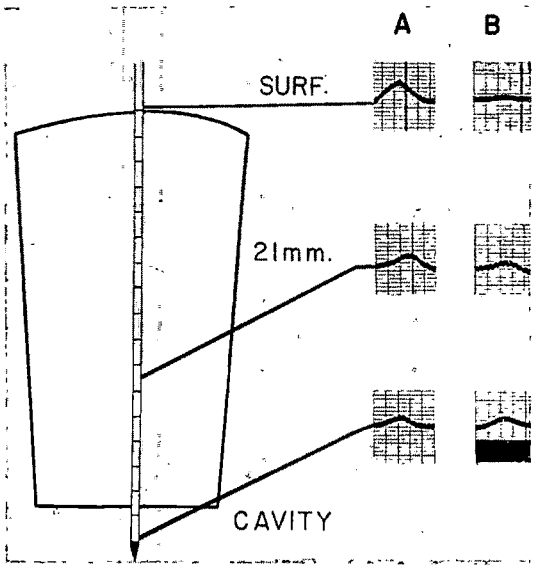


FIG. 4. Cavity, intramural and surface T waves. A, normal left ventricle; note the gradual increase in amplitude from cavity to epicardium. B, left ventricle in a case of mitral stenosis; note the gradual decrease in amplitude from cavity to epicardium.

ever, after the normal complex had been obtained, pressure was applied to the epicardial surface simply by pressing the electrode against the myocardium. This was done in order to determine the amplitude of the artificially produced injury current at the epicardium. It was found that pressure over the epicardial surface always gave rise to an extremely high injury current and a monophasic curve. (Fig. 7.) The amplitude of this type of injury current was far greater than any injury current registered from the intramural layers. Such injury current caused profound distortions in the configuration of QRS and T wave. The most striking alteration in QRS was a significant, sometimes sixfold increase in the amplitude of the R wave. T waves were usually incorporated into the monophasic curve.

TABLE I
HIGHEST INJURY CURRENT IN TRACINGS (MM.) FROM SUBENDOCARDIUM AND SUBEPICARDIUM

Patient	Subendocardium	Subepicardium
1	3	30
2	3	30
3	3	28
4	5	25
5	2	15
6	1	16
7	1	15
8	?	27
9	?	18
10	4	?
Mean	2.75 ± 0.46	22.6 ± 2.19

Time of Arrival of Impulse at Successive Myocardial Levels and Speed of Impulse Transmission. Since the introduction by Lewis[32] of the terms "intrinsic deflection" and "extrinsic deflection," it has been customary to use the former for time measurements. Activation of the muscle fibers situated immediately under a direct unipolar electrode is said by various authors to be represented by the onset, termination or the most rapidly inscribed segment of the intrinsic deflection.[33,34] For reasons stated elsewhere[35] and not germane to this discussion we have chosen the most rapidly inscribed portion as representative of activation of the myocardial fibers directly in contact with the tip of the plunge electrode and will refer to it hereafter as the "sharp segment." Furthermore, it did not make any significant difference whether the onset, termination or the sharp segment was used.

The time of appearance of the sharp segment of the intrinsic deflection as calculated against a constant time reference (e.g., onset of QRS in simultaneously recorded lead II) varies depending on how early in the process of depolarization the point of electrode contact is activated. The

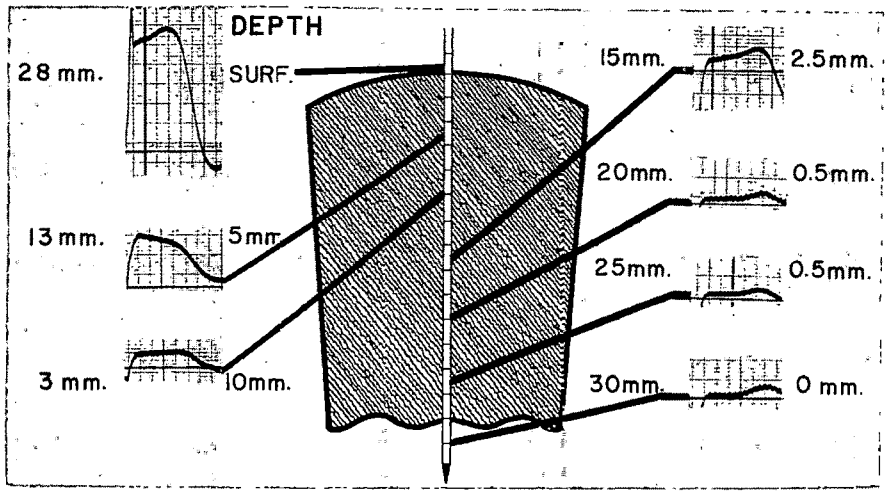


FIG. 5. Injury amplitude at various levels of human heart. Records obtained from all myocardial levels demonstrating absence of injury current in the inner layers between depths of 30 mm. and 15 mm. First evidence of injury current at 12 mm. It gradually increases to become of enormous magnitude at the epicardial surface. Note the concomitant alterations of the QRS complex in surface lead.

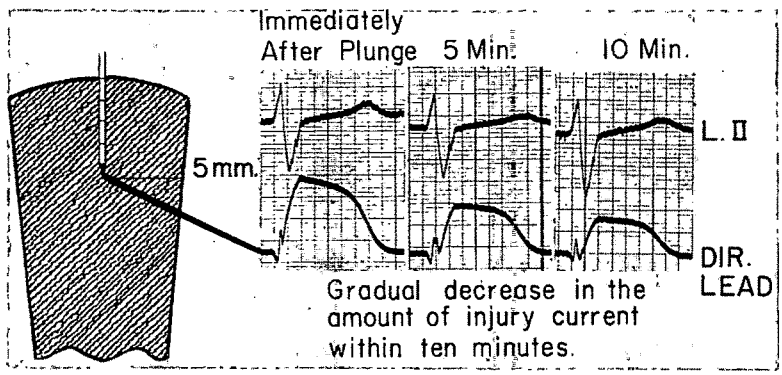


FIG. 6. Very high S-T segment immediately after introduction of electrode. Injury current decreases in amplitude when the electrode is allowed to remain in place for ten minutes.

sequence in which various points or layers of the myocardium are activated therefore can be determined by comparison of the appearance times of their intrinsic deflections. By employing this technic the time of arrival of impulse, that is, the time of activation of the various myocardial layers, was determined. Once the time of arrival of impulse at any two intramural levels with known depths was calculated, the speed of impulse transmission through that portion of the myocardium interposed between the two levels could be determined with ease.

As pointed out earlier in this communication, the subendocardial tracings of normal hearts yielded pure QS waves without very sharp intrinsic deflections. However, the QS waves obtained from the entirety of the subendocardial myocardium exhibited a constant time relationship to the onset of QRS in lead II. Lack of a detectable intrinsic deflection and also lack of a measurable time difference among QS complexes of the various subendocardial layers can be explained on the basis of two possibilities: either the speed of impulse through the subendocardial layers is too fast to be measured with the apparatus of the type used in this study, or depolarization of the myocardial fibers in these layers takes place simultaneously giving rise to electromotive forces that are oriented in multiple directions, hence cancelling each other out. It is suggested that both factors are operative; the high speed of activation causes the action

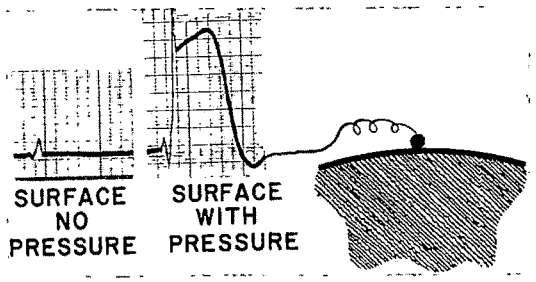


FIG. 7. The complex on the left shows surface potentials as recorded with a soft cotton-tipped electrode. The complex on the right illustrates potentials from the same point on surface after electrode is pressed against the myocardium. Note marked injury current; increase in R amplitude.

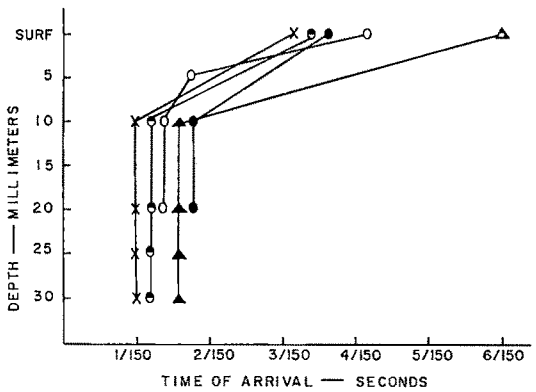


FIG. 8. Numbers on the ordinate represent depth of the myocardial levels in millimeters from which records were obtained in five cases. The numbers on the abscissa represent time intervals in seconds between the beginning of QRS in lead II and the intrinsic deflection in intramural and surface records. The intrinsic deflection of all myocardial layers between depths of 30 and 10 mm. appeared practically simultaneously, indicating a very rapid transmission of impulse through the inner layers. The impulse was relatively slow in the outer layers requiring $^{3}/_{150}$ to $^{5}/_{150}$ of a second to travel through the outer 10 mm. of the myocardium. These values may not be applicable in all instances due to the small number of cases studied.

potentials to be generated almost simultaneously and in multiple directions. The explanation may lie in the fact that, as has been suggested, the Purkinje system penetrates deeply into the subendocardium and conveys branches to muscle fibers present in this zone of the myocardium, hence instantaneous activation of all the muscle cells in contact with rapidly transmitting Purkinje fibers.

In the outer myocardial layers, on the other hand, the speed of impulse transmission ranged from 450 to 1,000 mm./sec. (Figs. 8 and 9.) Admittedly, measurements were extremely difficult in records taken from this portion of the myocardium due to the presence of injury current and obliteration by the injury current of the intrinsic deflections. However, the complexes obtained from the inner portion of the subepicardium, on the one hand, and those registered from the epicardial surface, on the other, displayed distinct intrinsic deflections and were suitable for the purpose of speed calculations.

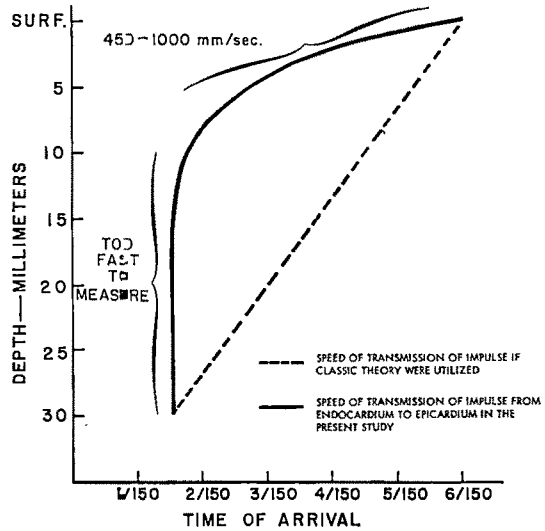


FIG. 9. Same cases as in Figure 8. The speed of impulse transmission through myocardial wall is calculated on the basis of time of arrvial of impulse to various myocardial levels. Note that while the speed in the inner layers is too fast to measure, it is of the order of 450 to 1,000 mm. per second in the outer 10 mm.

RIGHT VENTRICLE (ONE CASE)

A satisfactory intramural exploration could not be made in this case due to unfavorable exposure. The cavity tracing exhibited rS type of depolarization complex associated with a negative T wave. The only two intramural records obtained showed predominantly negative QRS complexes with marked S-T segment elevation which obscured the T waves.

COMMENTS

Comparison Between the Classic Theories and Findings in the Present Study. The prevailing concepts of electrocardiography are based on the assumption that the depolarization process begins in the endocardium and advances at a steady rate towards the epicardium. All layers of the myocardium are supposed to be equipotent in producing depolarization potentials, hence a steady increase in the size of the R wave

from endocardium to epicardium. (Fig. 10.) The direction of injury current according to available experimental and clinical data (Ref. 36 to 41 and others) is said to depend on the location of the injury within the myocardial wall. Thus subendocardial injury is supposed to cause

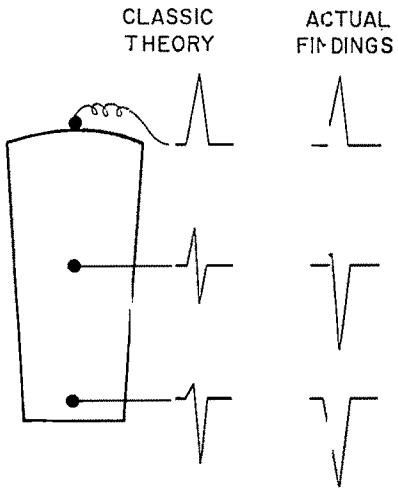


FIG. 10. Schematic representation of intramural and surface QRS complexes according to classic theory (left) and after our findings (right). Note that in contrast to classic theory, no R wave of any size was found in the inner layers. R wave begins at a considerable distance from the endocardium.

S-T segment elevation in leads recorded from the cavity and S-T segment depression in leads taken from the overlying epicardial surface; subepicardial injury is said to do the opposite, that is, produce S-T segment elevation on the surface and depression in the cavity. Furthermore, it is usually granted that all layers of the myocardium are equally capable of generating injury potentials.

The repolarization potentials are supposed to be equal in magnitude and directed opposite the depolarization potentials in any given myocardial cell or muscle strip. It has been inferred therefore that the T vector in a given segment in the myocardial wall is directed opposite the depolarization vector and that all layers are equally potent in generating repolarization forces. The data obtained in the present study are at variance in several respects with these classic theories.

1. *The inner layers of the myocardium do not appear to participate significantly in the genesis of the depolarization forces.* The subendocardial QS waves obtained in these experiments resembled cavity QS waves. The R wave was found to develop almost exclusively in the outer myocardial layers. In other words, the subendocardium was found to be "silent" insofar as the genesis of the R wave is concerned.

The notch observed on the descending limb of the subendocardial tracings in the two cases of mitral stenosis is not to be considered as representing subendocardial depolarization since it was present in the cavity tracings as well. Moreover, the time of occurrence of this notch was late, near the nadir of the QS. If it represented activation of the subendocardial myocardium it would have occurred early, indeed as the first component of the depolarization complexes registered from the subendocardial layers. It may be well to point out further that the amplitude of this notch did not exceed 1 m.v. in direct leads, hence was too small to be registered in the clinical electrocardiogram.

The speed of transmission of impulse through various myocardial layers was not uniform. Measurements made with the use of lead II as time reference showed the speed of impulse transmission in the subendocardial myocardium to be extremely rapid while in the outer myocardial layers it measured between 450 and 1,000 mm. per second.

2. *The reversed flow of current between the inner and outer layers described by Durrer and his associates*[42] *was not encountered in our studies.*

3. *The contribution to the genesis of injury potentials made by the subendocardial myocardium was found to be small.* The amplitude of the electrode-induced injury current in the subendocardial myocardium was negligible in comparison with similar injury current registered from the superficial myocardium. A monophasic curve was never obtained from the inner layers whereas it was a common occurrence in the outer layers. As illustrated in Table I, the mean value for amplitude of the injury current in the subendocardial myocardium was 2.75 mm. compared to the corresponding value of 22.66 mm. in the outer myocardium. Admittedly, the injury current in these experiments was that which was produced upon introduction of the plunge electrode into the myocardial substance, hence possibly different from injury current present in human myocardial infarction. However, there is reason to believe that the injury current present in spontaneously occurring myocardial infarction follows a similar pattern: on the one hand, injury current in experimentally produced myocardial infarction in dogs is generated in the same man-

ner as the electrode-induced injury current in this animal[43] and, on the other, there is a striking similarity between the electrophysiologic behavior of the human and dog's heart.

In most of the studies of intracavity electrocardiography in which a catheter electrode was used (Ref. 11, 18, 19 and others) a marked S-T segment elevation was registered when the catheter exerted pressure against the heart. This finding may be interpreted by some as evidence that the injury potential is produced in the subendocardial myocardium. We would like to point out, however, that a likely explanation for this discrepancy is that the catheter exerts pressure with each systole against the papillary muscles which have been shown to be capable of producing marked injury potentials. It is also possible that by jarring continuously against the myocardium the catheter electrode causes significant damage to the myocardium. These factors do not exist when the plunge electrode is used since, first, the papillary muscles can be avoided by proper selection of the site of exploration and, second, the electrode which is placed in the myocardial wall moves with it rather than against it; hence no jarring.

4. *T waves were identical in the cavity, throughout the myocardial thickness and on the ventricular surface in the majority of our cases.* In nine cases there was no gradient of potential across the myocardial wall insofar as the repolarization process was concerned. In one case of normal heart T waves showed a tendency to increase in amplitude in the endoepicardial direction. In one case of mitral stenosis the reverse phenomenon was found. We are cognizant of the fact that surface T waves may and often will change direction and amplitude upon exposure of the epicardial surface to room air, hence surface T waves obtained in the present study may not have been the true representation of the repolarization process as registered at the epicardial level; however, we have reason to believe, on the basis of our animal experimentations,[44] that exposure of the epicardial surface does *not* alter subendocardial and intramural T waves. The fact remains therefore that human cavity and intramural T waves are equal in magnitude in the majority of cases, a finding which is at variance with the classic concepts of electrocardiography. In most of the studies in which the left ventricular cavity was explored with the use of an intracardiac catheter electrode in human subjects[17–21] the T waves were found to be negative; however, in none of these cases was the epicardium explored by the direct method, hence no information was obtained concerning the direction of the T wave on the corresponding epicardial surface. While it is not impossible that the T waves in these cases were also negative at the epicardial surface, we do not believe that our data concerning T waves in this study are sufficiently conclusive to permit us to engage in the discussion of these discrepancies. Moreover, the general anesthesia used in subjects of the present study may have conceivably altered the vulnerable process of repolarization, another factor which may limit the value of our findings in respect to the T waves. According to classic theories, one would expect to find T waves of opposite directions in the cavity and on the epicardial surface and transition forms within the wall. In our studies, however, a biphasic T wave was never encountered in intramural leads. Furthermore, in experimentally produced myocardial infarction in dogs no significant abnormality was found in the intramural T waves recorded from the deep ischemic zone; T wave inversion appeared to be accounted for by repolarization changes of the outermost myocardial layers. By reason of the analogy found between experimentally produced infarction in dogs and spontaneously occurring infarction in humans, it is conceivable that the T wave inversion in human patients also represents repolarization aberrations of the outer myocardium and that ischemia of the deep myocardial layers may not alter T waves appreciably.

If our views concerning the similarity between experimentally produced infarction in dogs and spontaneously occurring human myocardial infarctions are valid, then our findings in the study of the former[35,43–48] will be applicable to the latter. Accordingly, old infarction limited to the subendocardial region will not be expected to alter the QRS complex. QRS changes will occur only when the superficial myocardium is involved. The presence of abnormal Q waves will indicate a lesion of the superficial myocardium. The presence of abnormal QS waves will represent through-and-through infarction with complete transmural death of the involved portion of the myocardium ("coronary QS waves") or transmural lesion interspersed with islands of living tissue ("mural type QS waves"). Subendocardial injury will not cause significant S-T segment displacement in the epicardial leads as the injury potentials generated in this

portion of the myocardium are too small to be registered. Subepicardial injury, on the other hand, will always be associated with marked S-T segment elevation on the corresponding surface. No T wave changes will be encountered in subendocardial ischemia since ischemia of this zone is not accompanied by significant repolarization aberrations. T wave changes will be accounted for by ischemic changes of the superficial myocardium.

An interesting finding in dogs which may have a counterpart in human beings is the type of S-T segment depression recorded at the epicardial surface and apparently generated in the superficial myocardium.[48] This type of S-T segment depression, which may be termed "primary epicardial," is distinguishable from the so-called reciprocal S-T segment depression by the fact that it is not due to S-T segment elevation on the opposite wall. It is an isolated finding which usually occurs at or near the margin of subacute or old infarcts. It is unlikely that this type of S-T segment depression is reciprocal to subendocardial injury as the latter has been shown not to generate sufficient injury potentials. Moreover, plunge electrodes placed in the subendocardium subjacent to the area of "primary epicardial" S-T depression failed to show the presence of S-T elevation in the subendocardium. The mode of genesis of this S-T depression is not known; however, its occurrence commonly near a subacute infarction speaks in favor of its being due to minor circulatory changes giving rise to biochemical alterations in the subepicardial myocardium.

This type of "primary epicardial" S-T depression, if substantiated in human subjects by more direct studies in the future, may explain the S-T depression found in angina pectoris and coronary insufficiency. Indeed, our findings concerning the relative silence of the subendocardium in generating injury potentials make one wonder how S-T segment depression of angina pectoris and coronary insufficiency could be accounted for by potentials generated in the injured subendocardium. It is suggested tentatively that S-T depression of angina pectoris and coronary insufficiency is most likely accounted for by injury potentials generated in the superficial myocardium as a result of ischemia or other changes.

Discrepancies Between Clinical and Electrocardiographic Findings. If all layers of the myocardium contributed equally to the genesis of electrocardiographic deflections then injury or necrosis of any layer, whether subendocardial or subepicardial, would alter the electrocardiogram; the degree of electrocardiographic alteration would depend on the thickness of the involved layer. However, certain experimental and clinical observations are incompatible with this concept of "electrical equipotence" of electrocardiography. Thus, Boyd and Scherf[49] in 1940 noted that traumatic injury to the subendocardial myocardium produced few electrocardiographic changes while surface lesions altered the tracing profoundly. Kisch[50,51] and Kisch, Nahum and Hoff[52] observed that with the use of KCl as the injurious agent, subendocardial injury produced very little S-T segment changes while application of quantities of KCl incomparably smaller than those injected into the subendocardium gave rise to marked S-T changes. Pruitt, Barnes and Essex[53] experienced difficulty in producing electrocardiographic changes by injuring the subendocardial myocardium. Thus there was prior evidence that the subendocardial myocardium did not produce as much injury potentials as did the subepicardial myocardium. To our knowledge, comparable information concerning the relative contribution of these layers to the genesis of depolarization potentials was lacking before the advent of intramural exploration. The human myocardium in its intramural layers had not been approached; the theories concerning the spread of activation through the human myocardium were largely inferential.

Clinically, however, Levine and Ford in 1950[54] reported six cases of subendocardial infarction, with autopsy confirmation, in which the electrocardiogram had shown no QRS changes. We have encountered at postmortem examination two cases of healed subendocardial infarction in which an electrocardiogram taken prior to death had failed to show QRS changes. QRS changes are rare in inflammatory processes involving the subendocardial myocardium, such as occur in some infectious endocarditides and in the condition known as cardiovascular collagenosis with parietal endocardial thrombosis,[55] in the latter disorder necrosis extends into the myocardial substance without giving rise to QRS changes. Similar discrepancies are encountered in chronic processes involving the subendocardial myocardium, such as endocardial fibroelastosis and old subendocardial infarction. In endocardial fibroelastosis, fibrosis

encroaches upon a significant portion of the subendocardial myocardium, causing intractable congestive heart failure and death, yet in most of the reported cases the QRS complexes remained unchanged.[56,57]

In contrast to failure of the electrocardiogram to reveal subendocardial damage, changes involving the subepicardial myocardium are almost always associated with significant alterations in all components of the electrocardiogram. Acute pericarditis gives rise to marked S-T segment and T wave changes; chronic constrictive pericarditis encroaching upon the superficial myocardial fibers reduces the amplitude of the R wave.[58–60] Levine and McLemore,[61] in an analysis of twenty-six cases of surgically proved chronic constrictive pericarditis, found marked QRS changes "quite diagnostic (four cases) or compatible with or suggestive (eight cases) of old myocardial infarction." We believe, on the basis of our experimental work in animals and human subjects, that the explanation for the marked QRS changes seen in some cases of chronic constrictive pericarditis is the extension of fibrous tissue into the outer myocardial shell which is normally responsible for the genesis of R potentials. If one compares endocardial fibroelastosis with constrictive pericarditis, both of which invade the adjacent myocardium, one is struck by the fact that the former produces little or no electrocardiographic changes whereas the latter is always accompanied by significant electrocardiographic alterations. These observations can be explained by the findings in the present study in which normal superficial myocardium was found to be predominantly responsible for the genesis of electrocardiographic deflections in general, and the R wave in particular, while the subendocardial myocardium was virtually "silent" from the standpoint of generating electrocardiographic forces.

General Clinical Applications. It is to be pointed out at the outset that the investigations on which this presentation is based were direct studies performed on the heart, as were the studies of the normal electrocardiogram and those after experimental production of myocardial infarction in dogs. No attempt was made to correlate the deflections so obtained with those recorded from the body surface in the same subjects. The studies were intended merely to examine, by the most direct method heretofore employed, the genesis of electrocardiographic deflections. The complexes obtained at the body surface represent, according to Wilson,[33] "the potential difference over the epicardial surface that would exist if the heart were to be completely exposed and surrounded by air." Undoubtedly, however, the intramural potentials contribute to those registered at the epicardial surface. Since the latter is the only part of the heart which is in direct contact with the surrounding tissues, it is not illogical to state that the deflections registered on the body surface are determined exclusively by the potential differences present at the epicardial surface.

To what extent the epicardial surface potentials are transmitted to the body surface and what the relationship between epicardial surface and body surface potentials is remains far from clear. However, as Wilson[33] stated, "the potential variation of a given part of the precordium in this animal (dog) are similar in every respect to those of the underlying portion of the anterior ventricular surface" and "one may justifiably conclude from the results of such experiments that *within certain limits* the principles that apply to the interpretation of the ventricular complexes of unipolar direct leads also apply to the interpretation of the ventricular complexes of the unipolar precordial leads." Therefore our findings concerning the silence of the subendocardial myocardium and predominance of subepicardial myocardium are within certain limits directly applicable to clinical, particularly precordial electrocardiograms.

This is true apparently because of the relative proximity of the precordial chest wall to the heart and especially to its anterior wall, on the one hand, and more favorable conduction through the chest wall than through the lung tissue, on the other. The precordial leads, V_1 to V_4, are so close to the anterior surface of the ventricles as to be considered specific for lesions involving this part of the heart. Lesions involving the posterior aspect of the heart affect these leads to a much lesser degree since the distance between electrode positions V_{1-4} and posterior epicardial surface is several times greater than the distance between the positions V_1 to V_4 and the anterior epicardial surface. According to Poisson's Integral, the "potential of a given dipole as determined at a certain point within its electromotive field will vary in inverse relationship to the second (or third) power of the distance of this point from the center of the dipole."[33] It follows that while close or

"semi-direct" leads such as V_{1-4} are relatively specific for the subjacent myocardial wall, remote limb leads especially aVF reflect the totality of the heart because the distances separating these lead positions from all parts of this organ are practically equal. Leads intermediate between semi-direct and limb leads, namely lateral chest, posterior chest and abdominal leads, may be regarded as transitional insofar as their specificity for the subjacent myocardium is concerned. Consequently, unlike the precordial leads to which our findings are probably directly applicable, the limb leads may or may not reflect the phenomena occurring in a limited area of the epicardium. That is to say, while the presence of an abnormal electrocardiographic complex, for instance coronary QS wave representing a small infarct on the anterior myocardial wall, results in a similar finding in the precordial electrocardiogram in a significant proportion (if not all) of the cases, a coronary QS wave from an infarct of the same size on the diaphragmatic surface is less likely to cause QS waves in the left leg lead. Therefore, in applying the findings of this study and our previous direct studies on dog's heart to the clinical electrocardiogram it may be well to bear in mind that the findings are most directly applicable to the "semi-direct" leads; the applicability decreases as the distance between the lead position and the heart increases. To be sure, this rule holds true for all the studies performed directly on the heart, a fact that has long been recognized.

Diagnosis of Subendocardial Infarction. On the basis of the facts and deductions outlined in the foregoing paragraphs we have set up and used certain criteria for the clinical diagnosis of acute and old subendocardial infarction. We have made this diagnosis in a group of patients in the past three years. Two patients of this group have died and postmortem examinations proved the diagnosis to be correct (the case histories will be the subject of a future report). It is for this reason that we feel justified in presenting our criteria as a working basis for the clinical diagnosis of subendocardial infarction.

Acute subendocardial infarction: This diagnosis may be made when such clinical symptoms of myocardial infarction as typical pain with evidence of tissue necrosis with or without congestive heart failure are associated with normal QRS complexes provided that adequate exploration of the chest and abdomen is performed. Admittedly, infarction Q waves may fail to show if a through-and-through infarction involves only a small portion of the epicardial surface; however, it is believed that an infarction large enough to give rise to clinical and laboratory evidences of tissue necrosis is more likely than not to cause abnormal Q waves. We have observed two patients with clinical symptoms of classic myocardial infarction in whom electrocardiograms showed S-T and T changes with normal QRS complexes. The diagnosis of acute subendocardial infarction was substantiated by evidence of tissue necrosis, namely, sustained elevation of white count, sedimentation rate and transaminase blood levels.[62] S-T segment depression and T wave inversion, when present, lend support to this diagnosis.

Not infrequently during the acute stage of subendocardial infarction the hemodynamic alterations propagate to the superficial myocardium and create a transient electrocardiographic picture indistinguishable from that of a through-and-through infarct, namely, infarction Q waves and S-T segment elevation. Under such circumstances the correct diagnosis can be made only in retrospect during the recovery stage of infarction. In this period the infarction Q waves disappear and QRS complexes return to normal as the superficial myocardium overlying the infarcted subendocardium regains its normal circulation. Here again one may argue that return of the normal QRS complexes may be due to "shrinkage" and reduction in size of the through-and-through infarct rather than the mechanism suggested. This argument is probably not valid inasmuch as the return of normal QRS complexes takes place long before fibrosis and "shrinkage" could be expected to have developed (within two to three days as seen in some cases).

Old subendocardial infarction: This type of infarction *per se* has no characteristic electrocardiographic representation, provided that conduction of impulse has not been interfered with. A diagnosis can be made, however, in the light of previous observations and a diagnosis of subendocardial location of the infarct during its acute stage. In some instances an extensive old subendocardial infarction can interfere with myocardial functions severely enough to give rise to progressive, intractable congestive heart failure. Therefore, although the diagnosis of a subendocardial scar in an asymptomatic patient may not imply important consequences,

it is of significant value in patients suffering from congestive heart failure. It is suggested that the diagnosis of old subendocardial infarction be entertained in any patient suffering from progressive congestive heart failure for which no obvious cause can be found and electrocardiograms show normal QRS complexes. This diagnosis is particularly apt to be correct if there is history of coronary pain suggestive of myocardial infarction.

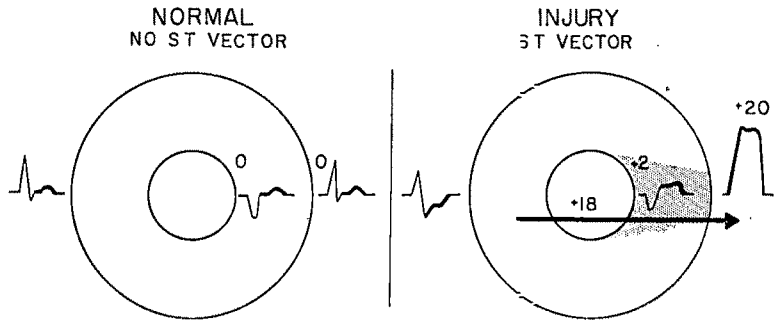


FIG. 11. Orientation of injury current vector. Diagrammatic cross section of the normal heart on left and heart with transmural injury on right. No S-T vector is present in normal heart. In the presence of transmural injury, there are more injury potentials in the outer layers than in the inner layers, hence an injury potential gradient. If of sufficient magnitude, this vector affects the opposite surface in a negative sense and creates S-T segment depression. The numbers used are arbitrarily chosen to indicate the relative magnitude of injury potentials. The dotted area represents injury.

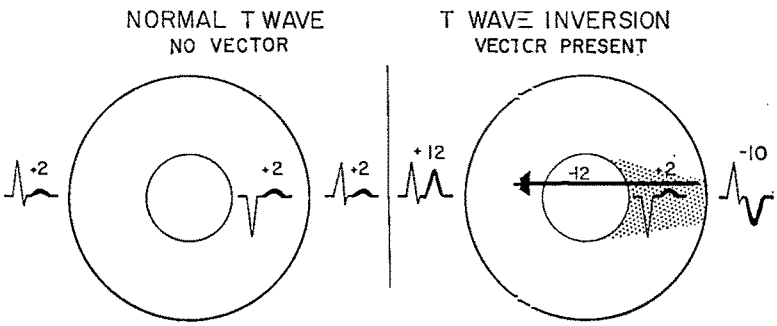


FIG. 12. Orientation of T vector, constructed on the same basis as Figure 11. Normal heart on left, heart with ischemic zone on right. Note that subendocardial T wave remains unchanged in spite of ischemia whereas surface T wave becomes deeply inverted, hence a T vector. Note also orientation of T vector and its influence on leads taken from areas overlying the ischemic zone and areas opposite ischemia. The dotted area represents ischemia.

The Question of S-T Vector. As already indicated, the injury potentials resulting from a given agent (introduction of the plunge electrode, through-and-through infarct) are far greater in the outer myocardial layers than in the subendocardial myocardium. There seems to be a gradual increase in the amplitude of S-T segment elevation from endocardium to epicardium. When plunge electrodes were placed at various points in many intramural layers in the entire zone of experimentally produced through-and-through infarcts in dogs, it was found that the pattern just mentioned in relation to relative magnitude of injury potential was present over the entire area deprived of its blood supply. In other words, it appeared as if the myocardial wall in the injured zone was constructed of innumerable sheets of muscle superimposed one upon another, with a progressive increase in the amount of injury potentials contained in those sheets. It is conceivable that the same is true in human myocardial infarction. Under such circumstances there will be potential differences between the adjacent sheets of the myocardium, thus giving rise to a vector force directed towards the surface of greatest positivity, that is, the epicardial surface. (Fig. 11.) Leads taken from areas overlying such epicardial

surface yielded S-T segment elevation and leads taken from the opposite surface showed reciprocally depressed S-T segments.

A similar statement may be made in relation to the T wave negativity. If the degree of T wave negativity in human myocardial infarction increases progressively from endocardium to epicardium, as was found to be the case in some cases of experimentally produced through-and-through infarct in dogs, then by employing the same argument as that relating to the injury forces, it may be inferred that the vector accounting for negativity of the T waves on the area of infarct is produced by repolarization differences that exist between the adjacent myocardial layers within the ischemic zone. (Fig. 12.) Such a vector, directed away from the ischemic epicardium and toward the ischemic endocardium, accounts for the increase in positivity of the T waves recorded over the opposite wall.

SUMMARY AND CONCLUSIONS

Intramural electrocardiography was performed in twelve patients subjected to thoracotomy for treatment of various intrathoracic abnormalities. The left ventricle was studied in eleven cases and the right ventricle in one. The procedure was found to be completely devoid of hazard.

On the basis of our observations and analysis of our data, the following conclusions seem to be justified:

1. Tracings obtained from the subendocardium exhibit pure QS waves; the positive component of the depolarization complex is generated wholly or almost wholly in the outer myocardial layers. The subendocardium therefore appears to be electrocardiographically silent in respect to the genesis of positive depolarization potentials.

2. The "electrocardiographic silence" of the subendocardium explains why QRS complexes are not altered in pure subendocardial infarction and other conditions involving this portion of the myocardium. Pathologic conditions involving the epicardial portion of the myocardium, such as inflammatory pericardial effusion and constrictive pericarditis, lower the amplitude of the R wave. Endocardial fibroelastosis encroaching upon the subendocardial myocardium and producing a situation comparable to constrictive pericarditis (the difference being in the location of the fibrous lining) causes very little if any QRS changes. No clinical or prognostic inference can be drawn from the degree of R wave changes; rather prognosis must be based on the composite clinical picture.

3. The speed of impulse transmission through the human myocardial wall is not constant. It is found to be very fast in the inner layers (the "silent zone") and of the order of 450 to 1,000 mm./sec. in the outer layers.

4. The subendocardium is also shown to be "sluggish" in generating injury potentials. While a given type of injury, for example injury produced by introduction of the plunge electrode, in the outer layers causes very marked S-T segment elevation, the same injury fails to produce any significant displacement of the S-T segment in the deeper layers. It follows from this and from the animal experiments done in this laboratory that profound injury to the subendocardial myocardium resulting from a variety of conditions, such as coronary insufficiency, myocardial infarction or certain types of acute endocarditis, does not give rise to significant quantities of injury potential, hence little or no S-T segment displacement. Injury to the outer myocardial layers, on the other hand, produces marked S-T segment elevation such as that seen in through-and-through myocardial infarction and acute pericarditis.

5. It is suggested that S-T and T changes commonly found in subendocardial injury may be due to certain functional alterations that take place in the outer myocardial layers. The nature of these alterations has not yet been defined; however, it is possible that it is biochemical in nature, resulting from the hemodynamic changes ordinarily accompanying subendocardial injury. The S-T segment depression resulting from such biochemical changes is tentatively termed "primary epicardial S-T depression" to distinguish it from that type of S-T depression which is reciprocal to elevation on the opposite wall.

6. Under the conditions of these experiments T waves were positive in the cavity and intramural leads. Surface T waves were positive in the majority of cases and flat to inverted in occasional cases. There seemed to be no gradient of potentials between repolarization forces present in the cavity, intramural layers and the epicardial surface in the majority of instances.

7. The presence of a normal or almost normal electrocardiogram in the face of clinical

evidence of profound myocardial damage (acute or old) speaks in favor of subendocardial location of the myocardial lesion. Marked electrocardiographic changes, on the other hand, may or may not bear a quantitative correlation to the degree of myocardial infarction inasmuch as pronounced electrocardiographic changes can represent only minor subepicardial damage.

8. Caution must be exercised in inferring prognostic implications from the electrocardiogram alone. Consideration of the clinical picture remains of utmost importance.

9. Certain working criteria for the diagnosis of acute and old subendocardial myocardial infarction are suggested.

Acknowledgment: We wish to thank Drs. Lois Schwartz, Jean-Louis Borduas and Sol Rothman, and Mr. Chet Smith for their valuable assistance in the preparation of this manuscript.

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The Splitting of Heart Sounds*

A Spectral Phonocardiographic Evaluation of Clinical Significance

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In selected instances splitting of heart sounds as detected with the stethoscope may be useful in cardiac evaluation. Because of the several conditions which can closely simulate splitting of the valve sounds (S-1 and S-2), this finding can also be confusing; how specific it is, as far as indicating disease and type of disease, is often not certain. Spectral phonocardiography, for reasons to be described in detail, is a useful tool for the re-examination of this problem.

Splitting of heart sounds results from separation of individual components normally present. "Reduplication" (or "duplication") is an unphysiologic designation. There is obviously no echo phenomenon as is suggested by the term; the heart sound is not reproduced.

Advantages of Spectral Phonocardiography in Study of Splitting. Elsewhere[1-3] the principles of the method for displaying and analysing of cardiovascular sound by means of the Bell sound spectrograph have been described in detail. In brief, the method produces recordings in which time, frequency and intensity are each represented in detail. Time is on the abscissa as in most physiologic tracings. The ordinate in these displays, unlike conventional oscillographic phonocardiograms, is frequency, not intensity. Intensity is represented by degree of blackness in any given portion of the recording. A frequency span to 750 cycles per second has usually sufficed.

The electrocardiogram and the respiratory mark have been used for timing purposes in these studies. The importance of recording respiration in any study of splitting of heart sounds resides (1) in the necessity of differentiating normal inspiratory splitting and (2) in the opportunity for identifying the basis of the split afforded by the effects of respiration upon splitting.

The spreading of frequency results in at least three advantages of spectral phonocardiography, the first of which is of primary pertinence to this particular study: (1) Identification of the separate constituents of the heart sounds is facilitated when the frequency spectrum is spread out because the constituent parts of the sound tend to show separation at some frequency levels, if not at other or all levels. For example, merging of the components at the lower end of the frequency scale tends to be the rule because of the high intensity of the vibrations in that range; on the other hand, higher in the frequency scale where intensities are less great the separate parts are more likely to be distinguishable. (2) Quality (timbre) is displayed and given physical definition. This property of the spectral phonocardiogram was used in a study of musical murmurs published elsewhere.[4] (3) The great dynamic range of cardiovascular sound—the intensity range from faintest to loudest sounds in the same patient and at the same auscultatory locus—is better encompassed[5] with simultaneous display, for example, of a very loud murmur and a very faint one in more nearly their true proportions.

Figure 1A shows the demonstration of splitting of heart sounds by this method. Filter system C (with passbands of intermediate width) is the one used in most of these studies since it appears to provide satisfactory simultaneous resolution in both time and frequency.[1] Even better time resolution (with some sacrifice of frequency detail) can be attained by using a filter system with wider passband characteristics (filter E). In the recording with the wider filter system E the individual components of the second sound can be identified in every cycle.

Splitting of heart sounds involving an interval of as little as 0.01 second between constituent

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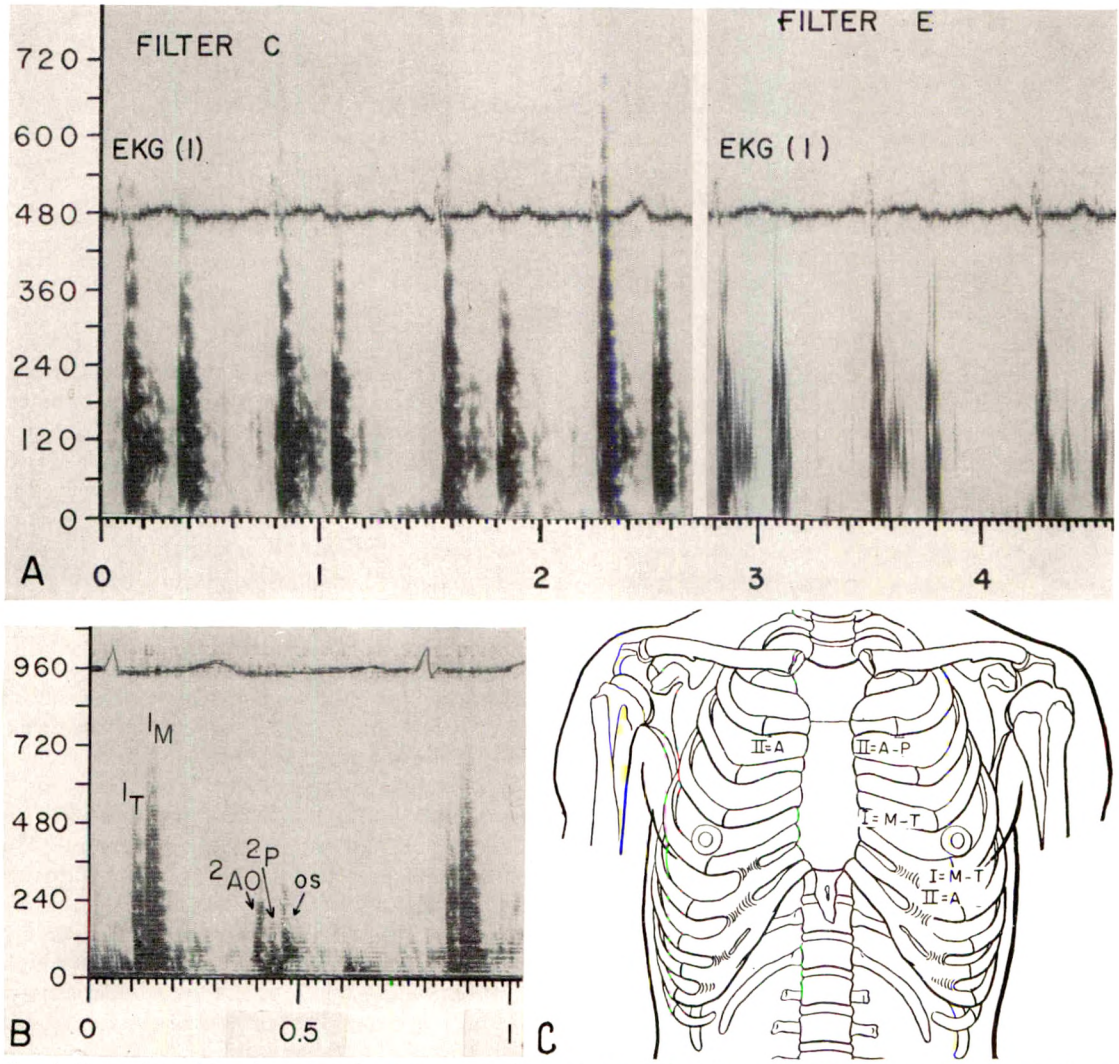


FIG. 1. General considerations. A, pulmonary area in fourteen year old patient with active rheumatic fever. Analyses with intermediate width of filter system (C) and with wide passband filter system (E). B, artefactual splitting of filter origin. Both the tricuspid closure sound (T) and the mitral closure sound (M) appear to be split. C, schematic representation of the normal constitution of the heart sounds (after Leatham).

elements is demonstrable by spectral phonocardiography. Elsewhere[6] we have presented analyses of the sounds produced *in situ* by the Hufnagel ball valve placed in the aorta for the treatment of aortic regurgitation. Although there is only one ball in the artificial valve, it impinges on three corners in opening. Splitting of the opening sound may result from the ball first striking one corner, then the others. Similarly in closing, although the ball becomes impacted on a circular seat, it may well strike one point of this circle, then complete the closure.

Figure 1B presents a source of artefactual splitting in spectral phonocardiograms. If the amplification of the signal put through the filter system is excessive one gets two peaks from each sound. In essence the filter system is activated twice, when the signal first hits it and again during decay of the signal. The recording was made from the apex in a patient with mitral stenosis. True splitting of the first heart sound occurred due to delay of the mitral closure sound. Also both the tricuspid and the mitral closure sounds are split. That the latter splitting is artefactual is suggested by the exact duplication of the harmonic pattern in each of the split components.

The experience reported here is selected from

a total experience with about 700 cases of heart disease, mainly valvular or congenital. In addition about thirty normal subjects have been studied.

Constituents of Normal Heart Sounds. Prior to a discussion of split heart sounds should be a consideration of the constitution of the normal sounds. (Fig. 1C.) The studies of Leatham,[7] using oscillograms recorded simultaneously from two or more areas, have done much to elucidate this subject.

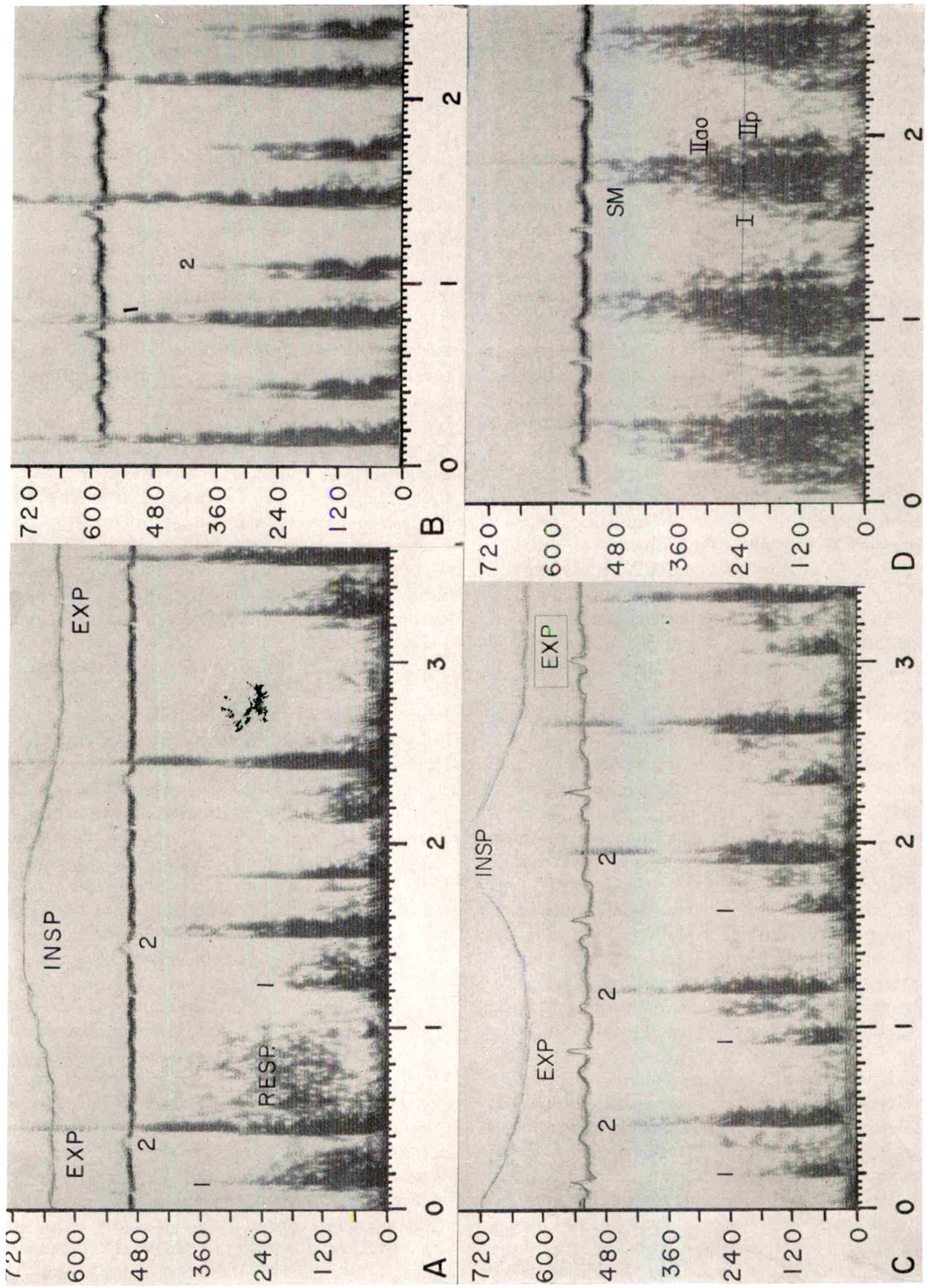
It appears that the normal second aortic sound is almost exclusively the result of aortic valve closure. On the other hand, the pulmonary second sound is contributed to by closure of both the pulmonary and the aortic valves. In most normal subjects inspiration results in a splitting of the pulmonary second sound. (Fig. 2A.) This is a result of delay in pulmonary valve closure as a consequence of increased right ventricular stroke volume, that is, it is a variety of "mechanical splitting" (*v. seq.*) resulting from a discrepancy in the stroke volumes of the two ventricles. The double origin of P-2 has adequate anatomic basis in the proximity of the aortic and pulmonary valves, one behind the other, slightly to the left of the sternum. Knowledge of the double origin of "P-2" removes the confusion which otherwise is likely to be occasioned by such findings as "P-2 louder than A-2" in systemic arterial hypertension.

The first sound in the tricuspid and mitral areas has contributions from the closure of both sets of atrioventricular valves. Again, in most normal subjects slight splitting of the first sound, especially in the tricuspid area and especially in expiration, is demonstrable. When such splitting occurs the mitral closure is usually the first of the two constituents. The second sound at the apex has its origin almost exclusively in closure of the aortic valve. As a generalization, aortic events are well transmitted to the apex, at any rate they are better transmitted than pulmonary events.

Asynchronism in the ejection of the two ventricles has been demonstrated in the past in both animals[8] and man.[9] Electrokymography is a technic recently applied to this problem. Comparing the onset of the major ascending limb of the carotid pulse with the corresponding point on the pulmonary artery electrokymogram, Ellinger and associates,[10] in sixty-eight normal young adults, found that ejection from the left ventricle began first in thirty-three subjects by .01 to .03 second, from the right ventricle first in twenty-one subjects by a similar interval, and from both ventricles synchronously in the remaining subjects. On the other hand, using a similar method Luisada and Fleischner[11] found right ventricle ejection had precedence in all of eight normal subjects by .025 to .03 second. Such demonstrations of asynchronism of the onset of ejection cannot be translated directly to time of closure of the atrioventricular valves. Because of higher diastolic pressure in the aorta than in the pulmonary artery isometric contraction (after closure of the atrioventricular valves) might be expected to be longer in the left ventricle than in the right. By such a mechanism it is possible that mitral closure might slightly precede tricuspid closure and still onset of ejection from the left ventricle would occur later than that from the right. Some studies[12] indeed suggest that isometric contraction is longer in the left ventricle. On a rational basis, precedence of tricuspid closure over mitral closure might be anticipated since the right atrium contracts before the left, and by the time the ventricles contract the tricuspid leaflets have had time to reach a slightly more closed position than the mitral.

Conventional oscillographic phonocardiography has purported to show contributions to the heart sounds of several origins in addition to valve closure. It is best to consider the enumeration of these factors as a catalogue of possible sources of contributions. It is difficult to imagine that what is scarcely at the most more than two or three lines in an oscillogram can be picked out and indicated as arising in this or that physiologic event. The evidence for significant contribution of some of these factors is not convincing. Some of the alleged components may, however, be exaggerated under pathologic conditions as, for example, the ejection vibrations in arterial hypertension and dilatation ("early systolic click") and the opening sound of the atrioventricular valves in the presence of fibrosis of those structures (mitral and tricuspid "opening snap"). The contributions, possible and certain, to each sound can be listed in this manner:[13]

1. First heart sound
   - A. "Atrial components": vibrations in the ventricle in response to atrial contraction
   - B. Closure of the atrioventricular valves
   - C. Vibrations of the contracting myocardium


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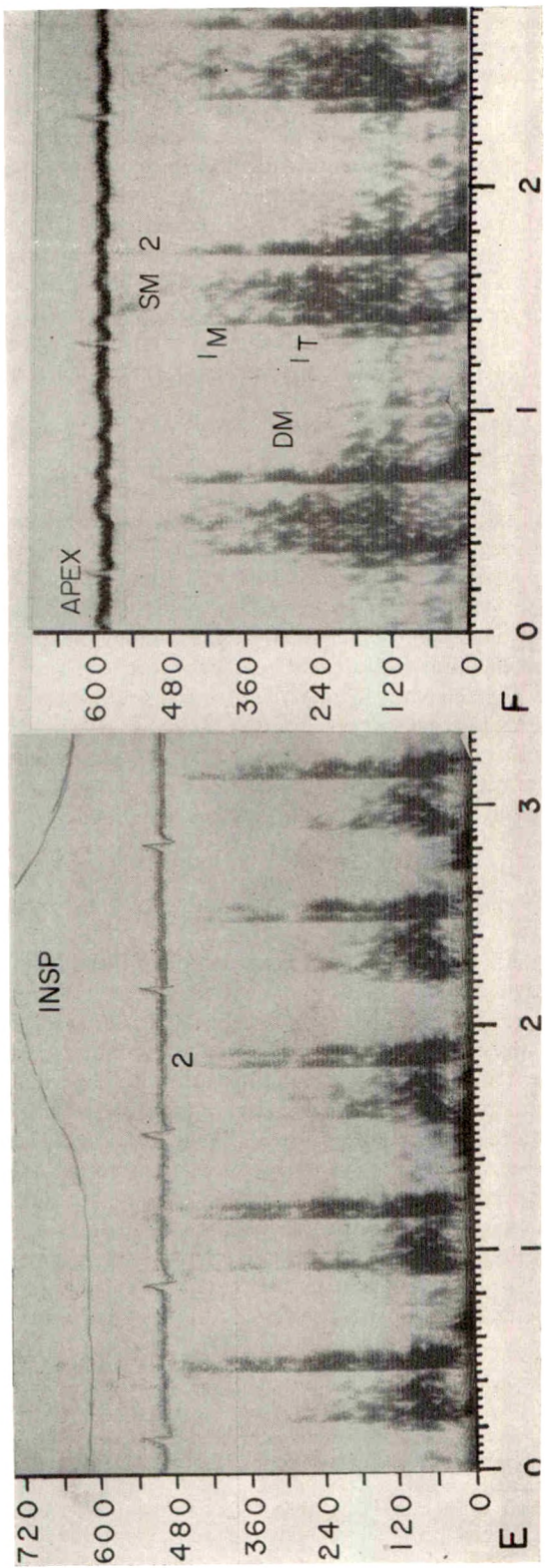


FIG. 2. Mechanical factors in the splitting of heart sounds: A, pulmonary area in thirteen year old child; slight early systolic murmur. B, apex in forty-three year old patient with interatrial septal defect. Each component of split S-2 has frequency pattern of a valve-closure sound. C, pulmonary area in fifty year old patient with systemic arterial hypertension and left-sided thoracoplasty for pulmonary tuberculosis. Split P-2 probably due to exaggerated negativity of intrapleural pressure. Scratchy mid- or late systolic murmur believed to be extracardiac. D, pulmonary area in pulmonary stenosis, probably valvular type; note "Christmas tree" ejection stenosis murmur and delayed pulmonary closure sound or "pulmonary reversal snap" (up). The systolic murmur appears to continue slightly after the aortic closure ("nao"). E, pulmonary area in twenty year old patient with mitral regurgitation. There is splitting of the second sound which is exaggerated by inspiration; note the decrescendo systolic murmur. F, apex in a patient with rheumatic mitral regurgitation. The systolic murmur begins immediately with the second component of the split first sound which therefore is probably mitral closure sound. If so, it is considerably delayed and has an anomalous relationship to the tricuspid closure sound. The second sound is unitary—presumably only aortic closure sound—and is followed immediately by a decrescendo diastolic murmur which probably had its origin at the aortic orifice.

D. Opening of the arterial valves
E. Ejection vibrations

II. Second heart sound
A. Relaxation of the myocardium
B. Closure of the arterial valves
C. Water-hammer phenomenon in the arteries(?)
D. Opening of the atrioventricular valves

Factors Determining Audibility of Splitting in Heart Sounds. The interval separating the individual components is clearly the most important factor determining audibility of splitting but it is by no means the only factor. It is generally stated that an interval of .05 to .06 second is the minimum detectable by ear. Experience indicates that, when other factors (to be discussed) are favorable, splitting of lesser degrees (of the order of .03 second) can be detected.

The experiments of Helmholtz and of Mayer and Stumpf[14] concerning the greatest number of beats per second that can be heard has relevance in this connection. With pure tones 41 beats per second (interval of about .024 second) are audible at 96 cycles, 58 at 256 cycles (.017 second interval) and 107 at 575 cycles (.009 second interval). For the complex frequency combinations involved in heart sounds, the performance of the ear is much less favorable.

Training and experience are obviously important. When the first of the two split components is unusually accentuated and reverberating, fusion with the second component and reduction in audibility of the splitting results. (An example is the lesser audibility of normal inspiratory splitting of the second sound at the base when the aortic component is greatly accentuated in systemic arterial hypertension.) It is self-evident that splitting will less likely be detected when one of the components is of too low intensity or, what amounts to the same result, is of lower frequency composition than is easily appreciated by ear. Because of the physiologic properties of the ear, a very intense component will render the ear less capable of detecting a second component which would ordinarily be heard and which is separated from the first by an appropriate interval. When the second component is accentuated or when it initiates a murmur, splitting may be more evident.

Splitting of Heart Sounds in Normal Subjects. Splitting of the second sound at the base with inspiration was known a century ago. The classical studies of Potain[15] published in 1862 are particularly noteworthy. Such splitting is usually most striking in children. The normal effects of respiration on splitting provides a useful method for the bedside analysis of splitting in bundle branch block and in conditions simulating split. The normal splitting with inspiration is a result of the discrepancy in stroke volume of the two ventricles. Inspiration increases venous return to the right side of the heart. This is a physiologic counterpart of the increase in right ventricular stroke volume with resultant splitting of S-2 which occurs as a rule with the left-to-right shunt of interatrial septal defect. (Fig. 2A.)

Normal splitting of the second heart sound may be exaggerated in patients with a normal heart who have, however, increased inspiratory negativity of intrathoracic pressure as a result of respiratory disease. (Fig. 2B.) The presence of striking inspiratory splitting in later decades of life, without signs of heart disease, suggests respiratory disease such as inspiratory airway obstruction and pleural or parenchymal fibrosis. It is likely that in many types of heart disease a non-specific splitting of the heart sound with inspiration may result from reduced pulmonary compliance which in turn is secondary to pulmonary congestion or fibrosis.

CLASSIFICATION OF PATHOLOGIC SPLITTING OF HEART SOUNDS AND OF CONDITIONS SIMULATING SPLIT HEART SOUNDS

All true splitting of heart sounds results from asynchrony in closure of the valves on the opposite sides of the heart.* Instances of pathologic splitting fall into two main types which can be called after Leatham[16] electrical and mechanical. In cases of the first type, ventricular asynchronism is the result of delay in the activation of one ventricle relative to the other. Bundle branch block is the classical example and idioventricular pacemaker, as in ventricular premature contractions and complete heart block, another. In the so-called mechanical group the ventricular asynchronism

* Another mechanism for splitting of heart sounds, namely asynchronous closure of individual cusps of a single valve, as proposed by Skoda in 1853,[17] is unlikely as a basis for a significant degree of splitting. No corroboration for this idea could be obtained by examining frame-by-frame available motion pictures of functioning heart valves made at a speed of 24 frames per second. However, the minute splitting of the opening and closing sounds of the Hufnagel valve suggests the occurrence of a similar phenomenon in the heart.[6]

results from a discrepancy in the stroke volumes of the two ventricles as a result of inspiration or shunt or from a discrepancy in the rate of ejection from the two ventricles, as in aortic and mitral regurgitation. A most striking example of splitting of heart sounds of the mechanical type is that seen in the case of the second sound in

I. Those confused for split S-1:
   A. Normal atrial sound
   B. Presystolic gallop
   C. Protosystolic click
      1. Due to dilated pulmonary artery
      2. Due to disease of ascending aorta
      3. Due to pericardial adhesions

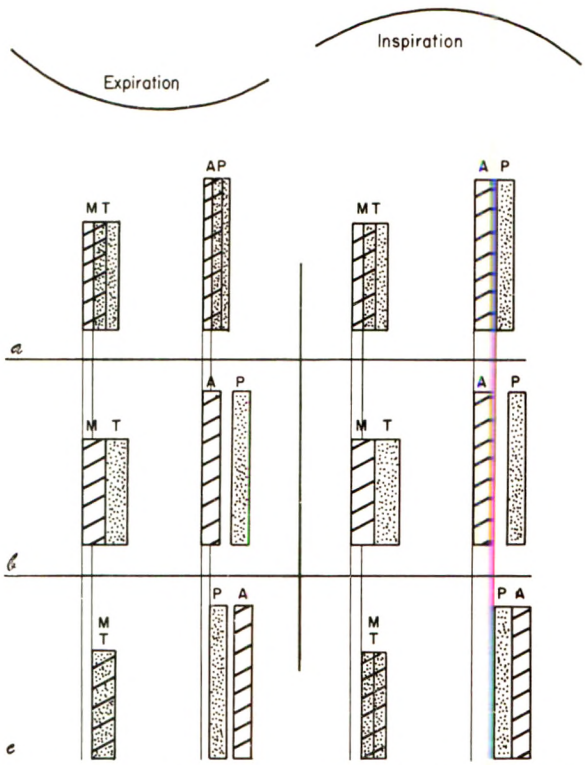


FIG. 3. Effect of respiration on the splitting of heart sounds in bundle branch block: (*a*) normal, (*b*) right bundle branch block and (*c*) left bundle branch block.

atrial septal defect. Here the stroke volume of the right ventricle is increased and the pulmonary closure sound delayed. Seemingly, systole is prolonged in the right ventricle relative to that in the left because of the larger amount of blood with which it is presented. (This is an exaggerated form of the splitting of P-2 which occurs with inspiration in most normal subjects.) The marked inspiratory splitting of the second sound which may occur in patients with an increased negativity phase of intrapleural pressure is another example of "mechanical" splitting.

Those adventitious sounds which may create a situation confused for splitting by the ear and, in some instances, by oscillographic phonocardiography can be classified in this manner:

II. Those confused for split S-2:
   A. Telesystolic click due to pericardial adhesions
   B. Mitral opening snap
   B. Normal third heart sound
   D. Protodiastolic gallop
   E. Isodiastolic pericardial snap of constrictive pericarditis.[19,20]

Bundle Branch Block. Ventricular asynchronism and resultant splitting of both heart sounds is to be expected in bundle branch block. In practice, splitting of the second sound occurs more commonly than splitting of the first sound. Furthermore, splitting of impressive proportions is more common in right bundle branch block than in left. Normally the mitral and

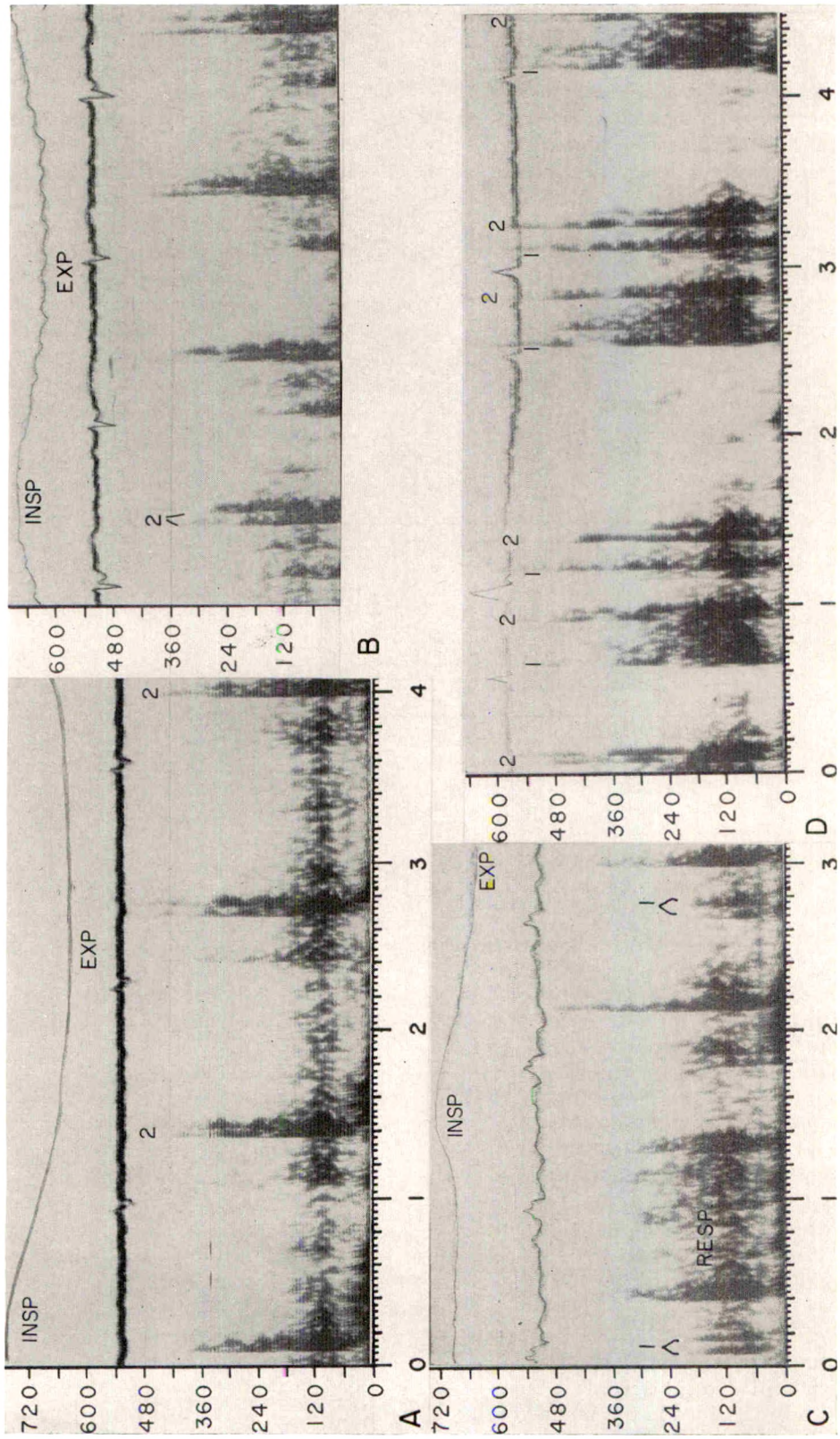


FIG. 4. "Electrical" type of heart sound splitting. A, pulmonary area, left bundle branch block; note paradoxical effect of expiration. B, pulmonary area, right bundle branch block; note exaggeration of splitting with inspiration. C, split first sound in left bundle branch block. Although not clearly split, the second sound is widest in expiration. D, mitral regurgitation; second sound split in normal cycles; both first and second sound split with ventricular premature contractions; note the decrescendo systolic murmur.

aortic closures usually precede slightly the closure of the tricuspid and pulmonary valves. Left bundle branch block is less likely to produce splitting because delay in the closure of the mitral and tricuspid valves tends merely to cancel this normal asynchronism. Right bundle branch block, on the other hand, exaggerates normal asynchronism. It is difficult to understand why the first heart sound (Fig. 4C) is not more frequently and markedly split in cases of bundle branch block than it is, especially since splitting of the first sound does occur quite regularly with ventricular premature contractions, a seemingly comparable situation.

The effect of respiration on the split second sound of bundle branch block is different in the case of the two types, left and right. (Fig. 3.) With inspiration the split is exaggerated in the case of right bundle block and decreased or eliminated in the case of left bundle branch block. These contrasting phenomena are obviously the result of interplay between the normal inspiratory splitting of the second sound and the splitting produced by the bundle branch block. Repeatedly, students informed of these differential points have been able to determine at the bedside the type of bundle branch block present.

When right bundle branch block and a protodiastolic gallop of left ventricular origin are present together, the second component of the second sound can be expected to occur closer to the gallop sound than in the absence of bundle branch block or with left bundle branch block.[18] This is clearly a differential point of less usefulness because of the relative infrequency of the proper combination of sounds and because the split second sound and the gallop tend to have maximum audibility in different areas, pulmonary area and apex, respectively.

These studies have not revealed "three well developed components of the first sound" in bundle branch block as has been said to occur.[18]

Illustrative cases of right bundle branch block are presented in Figures 4B and 5 and of left bundle branch block in Figures 4A and C.

Ventricular Premature Contractions. Although the first heart sound is not commonly split in bundle branch block such splitting does occur as a rule in ventricular premature contractions. This is a bit surprising since they would seem to be comparable situations. Possibly the fact that greater prolongation of the QRS occurs with premature contractions than with bundle branch

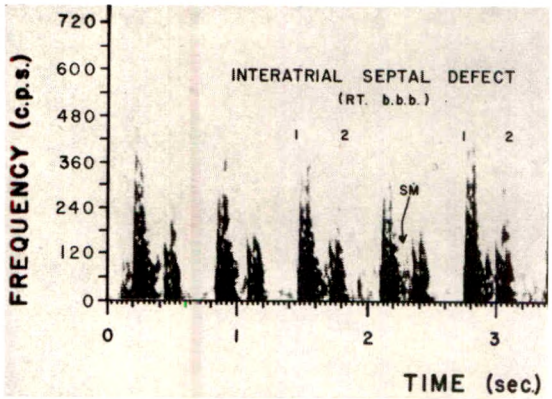


FIG. 5. Splitting of both the first and the second heart sounds in a patient with two mechanisms for splitting: right bundle branch block and interatrial septal defect.

block accounts for the seemingly conflicting results. Occasionally it is possible to localize the initiating focus to one or the other ventricle by comparing the intensity of the two components of each sound in the several areas. Figure 4D demonstrates splitting of both the heart sounds produced by a ventricular premature contraction. The patient also had rheumatic mitral regurgitation and it is of note that the second heart sound is split in normal cycles. This type of splitting is to be discussed in more detail later.

Shunts, Particularly Interatrial Septal Defect. Splitting of the second sound occurs in the great majority of cases of interatrial septal defect. (Fig. 1B.) This splitting appears to be the result of the discrepancy in stroke volume of the two ventricles. This is a situation comparable to the splitting of the second sound which occurs normally with inspiration and which is also the result of discrepancy in ventricular stroke volumes. When right bundle branch block is also present, the splitting is exaggerated. (Fig. 5.) Respiration has little or no influence on the splitting of the second sound in these cases.

In about half the patients with interatrial defect splitting of the first sound is also present. The splitting of the first sound must have a different basis than the splitting of the second sound. Prolongation of iso(volu)metric contraction cannot be the basis since by definition both the arterial and the atrioventricular valves are closed during this phase. It is possible that a delay in the tricuspid closure results from the fact that right up to the time the dilated right ventricle contracts it is still accepting blood and the tricuspid leaflets are spread to a maximum

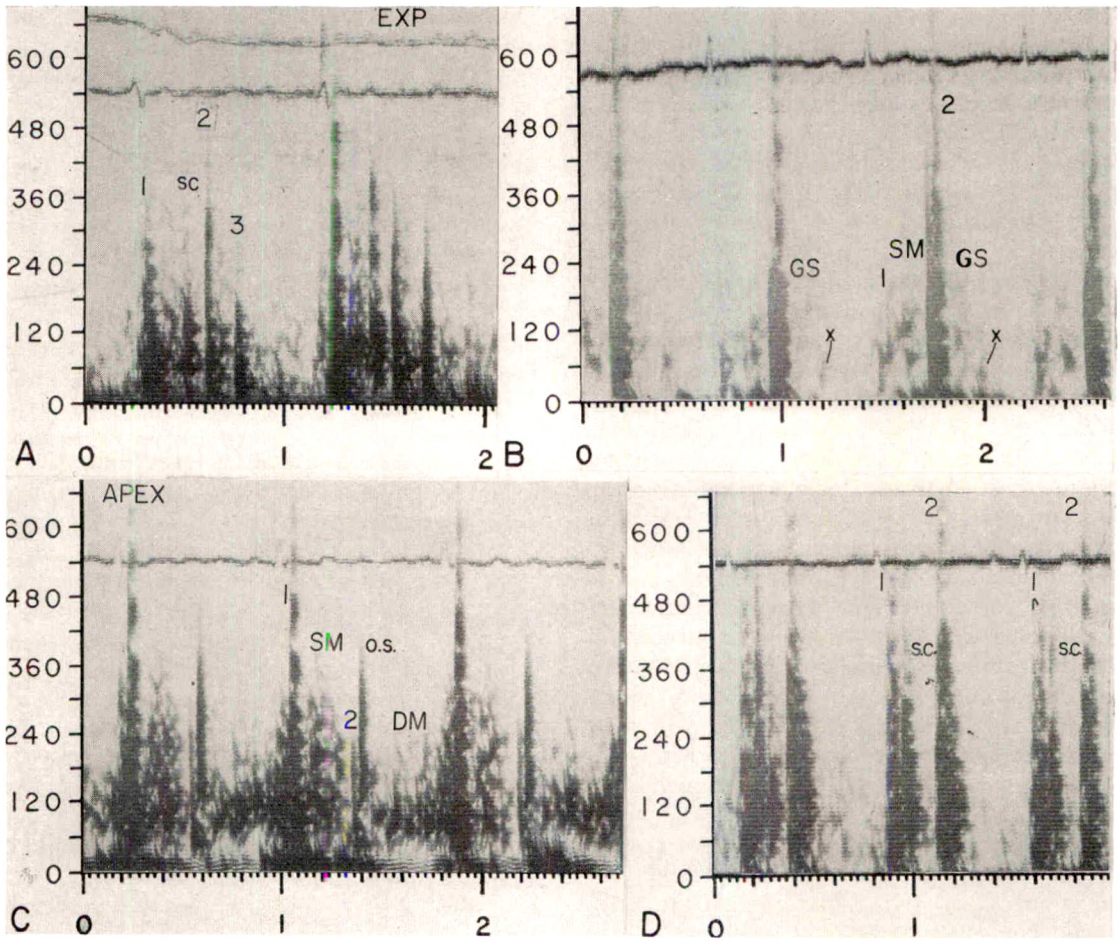


FIG. 6. Phenomena simulating split sounds. A, late systolic click of about the same intensity as seen at the apex was also present at left sternal border and pulmonary area. There is a history of acute rheumatic fever, during which pericarditis was observed, four months before this recording; third heart sound is also present. B, this patient with mitral stenosis had considerable pulmonary hypertension with resultant accentuation of the pulmonary closure sound. The temporal relationship of the two components of P-2 is not altered. There is an exceedingly subtle diastolic murmur (probably Graham Steell) beginning immediately with P-2. There is a faint sound in mid-diastolic (which is difficult to identify) at the termination of the Graham Steell murmur. It is too early for an atrial heart sound and perhaps too late for a third heart sound. It may represent a valve closure sound—slight leak occurs through a small separation of the cusps as long as the pressure differential is sufficient to maintain the separation. As soon as the pressure differential drops below a certain threshold, coaptation of the previously separated surfaces occurs with production of an audible sound. C, recording at the apex in a patient with severe mitral stenosis and a moderate degree of mitral regurgitation; note the snapping mitral closure sound, the systolic murmur and the diastolic murmur with presystolic crescendo. There is seemingly a split second sound. Actually it is clear that this is in fact a second sound followed closely by an opening snap. This patient also has aortic stenosis. The aortic closure sound is so attenuated that it is doubtful that it is transmitted to the apex where the pulmonary closure sound alone is represented. That the sound marked OS is indeed mitral opening snap is corroborated by its snapping appearance, pure frequency content and elevated frequency "bottom." D, an example of early systolic sound in the pulmonary area caused by dilated pulmonary artery due in this case to atrial septal defect. In addition both the first and the second sounds are slightly split.

degree. The left ventricle, on the other hand, because of its different pressure-volume characteristics will more likely have completed filling at the corresponding time and the mitral valve will be partially closed.

Since many cases of interatrial defect have an early systolic click on the basis of dilated pulmonary artery, this must be distinguished from splitting of the first heart sound.

Anomalous pulmonary venous return can be

expected to produce a splitting of heart sounds of the same type as that which occurs in interatrial defect. Our experience with spectral phonocardiography in this anomaly is limited. Patent ductus arteriosus, by increasing the stroke volume of the left ventricle over that of the right, should produce splitting of heart sounds. That splitting does not occur more commonly with patent ductus may be yet another instance of absent splitting with delay in the left-sided valvular events.

Variations in Arterial Pressure. It is the general impression that hypertension causes splitting of the second heart sound, and especially that pulmonary hypertension causes a split P-2. Phonocardiographic analysis does not corroborate this impression. Even clinical experience does not relate splitting of the second sound to systemic arterial hypertension. In pulmonary hypertension studies, very slight splitting was frequently demonstrated. However, the temporal relationship of aortic and pulmonary closure was not altered: pulmonary closure did not occur prematurely as a result of elevation of pulmonary arterial pressure. (Fig. 6B.) The pulmonary closure sound was, of course, accentuated in intensity and frequency span. With pronounced elevation of pulmonary pressure the sound was often prolonged and reverberating. The clinical impression of split P-2 with pulmonary hypertension has been engendered in large part by the easy audibility of the mitral opening snap in the pulmonary area of patients with pulmonary hypertension on the basis of mitral stenosis.

Contrariwise, hypotension in the pulmonary artery in congenital pulmonary stenosis can result in delay of pulmonary valve closure and splitting of the second sound. (Fig. 2D.) Since it is the pressure differential on the two sides of the valve that controls its closure, the effect of pulmonary hypotension is relatively greater by reason of the ventricular hypertension. When ventricular systolic pressure is in the range of 300 mm. Hg or even higher, it might be anticipated that a longer time will be taken for this pressure to fall below that in the pulmonary artery.

In cases of valvular pulmonary stenosis of the congenital type, the valve is as a rule merely a perforated membrane. Valve closure in the usual sense is inconceivable. The sound that is heard and recorded is probably a snap of this diaphragm toward the ventricle. This sound may be termed "pulmonary reversal snap."

A rough indication of the pulmonary pressure in cases of tetralogy of Fallot with infundibular stenosis can be obtained from the presence or absence of a pulmonary closure sound. Furthermore, after an adequate Blalock-Taussig operation, the pulmonary closure sound should become evident in such cases, providing pulmonary atresia is not present.

Valvular Heart Disease. Delay of the mitral closure sound is characteristic of mitral stenosis especially if atrial fibrillation is also present. (Fig. 1B.) This delay may be due largely to the elevation of left atrial pressure since the delay is greatest in cases with the greatest elevation of left atrial pressure. Splitting of the first sound is not more common in mitral stenosis than it is, due to the usual precedence of mitral closure over tricuspid closure and to obscuration of the tricuspid closure sound by the presystolic rumble in cases with sinus rhythm. (Fig. 6C.)

In mitral regurgitation splitting of the first sound of the same type, delay in mitral closure, is not infrequently demonstrable and the systolic murmur is found to begin immediately with the second component, the mitral closure sound. (Fig. 2F.)

Splitting of the second heart sound occurs commonly in severe mitral regurgitation. (Figs. 2E and 4D.) Presumably because of the double route of ejection from the left ventricle, aortic closure occurs prematurely. This splitting is likely to be obscured, however, by the systolic murmur and may be demonstrable only by graphic methods. Because of low diastolic pressure in the aorta and accelerated left ventricular ejection, S-2 may be split in aortic regurgitation.

Absent Pericardium. In our experience, splitting of the heart sounds is a frequent finding in patients in whom the pericardium has been removed for relief of constrictive pericarditis or for other reasons.

Clicks and Snaps. Early or late systolic clicks produce a situation easily confused with split first or second sound, respectively. The opening mitral snap (Fig. 6C) is equally easily confused with the second component of a split second sound. The genesis and clinical significance of systolic clicks are discussed in more detail elsewhere.[3,21] The most frequent cause of early systolic click is disease with dilatation of the ascending aorta or the pulmonary artery.

Pericardial adhesions may be responsible for telesystolic clicks as well as some protosystolic ones. The ear can often distinguish systolic clicks and opening snaps from valve sounds by their snapping quality. Similarly, the spectral phonocardiogram usually displays distinctive features in these added sounds: they usually have more homogeneous frequency content and often their "frequency bottom" is not at the baseline, as in the case of valve sounds. The presence of demonstrable splitting of heart sounds proper, in addition to the added sound, clinches identification of the click or snap on auscultation and in the spectral phonocardiogram. See Figures 6A, C and D for examples of clicks and snaps which create combinations of sounds simulating splitting.

Gallops. In gallops the degree of separation of the added sound from the heart sounds proper endows the rhythm with a cadence rarely encountered with true splitting of the heart sounds. When any question arises the relationship to the electrocardiogram will, in the case of the atrial gallop which precedes the QRS, identify the adventitious sound. Possibly more easily confused with a split second sound is the early diastolic sound, sometimes termed gallop, noted in constrictive pericarditis and also pericarditis with effusion.[19,20] This sound occurs closer to the second sound than does the protodiastolic gallop. (Fig. 6A.) "Isodiastolic pericardial snap" is Lian's term.

SUMMARY AND CONCLUSIONS

Normal and pathologic conditions resulting in splitting of heart sounds are reviewed. The influence of respiration on splitting of the second sound can assist in identifying the type of bundle branch block present and in distinguishing true splitting of heart sounds from simulating conditions. Arterial hypertension is not a cause of splitting of heart sounds. Asynchronism of valve closures is the fundamental basis for splitting of heart sounds and may have two general bases which, following the terminology of Leatham,[16] may be designated electrical (asynchronism due to abnormality in the activation of the ventricles, as in bundle branch block, ventricular extrasystoles and idioventricular rhythm) and mechanical (asynchronism due to discrepancy in ventricular stroke volumes, as in atrial septal defect, or in rate of ejection, as in mitral and aortic regurgitation). In a patient over forty years of age without other evidence of heart disease, striking inspiratory splitting of heart sounds suggests respiratory disease which is accompanied by exaggeration of intrapleural negativity of pressure during inspiration. Inspiratory airway obstruction as by bronchogenic carcinoma or asthma and parenchymal or pleural fibrosis are possibilities.

Early and late systolic clicks and the mitral opening snap are the conditions most likely to simulate splitting of heart sounds.

Because of much overlap of the degree of splitting with that occurring normally, splitting is of limited diagnostic value in the pathologic states with which it may occur: interatrial septal defect, bundle branch block, mitral or aortic regurgitation and respiratory disease resulting in exaggerated cyclical variations in intrapleural pressure. In general, however, splitting has more pathologic significance (1) when it occurs in adults than when it occurs in children, (2) when splitting is exaggerated with expiration rather than with inspiration (left bundle branch block), (3) when there is no clinical evidence of respiratory disease and labored respirations and (4) when splitting persists throughout all phases of respiration (as usually is the case in interatrial septal defect).

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Diagnostic Value of Phonocardiography in Mitral Stenosis*

Mode of Production of First Heart Sound

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IN 1911 Weiss and Joachim[1] reported a delay in the onset of the first heart sound in mitral stenosis. More than thirty years later Cossio and Berconsky[2] rediscovered this phenomenon using a more refined technic of phonocardiography. In 1951 Sprague and his associates,[3] unaware of either publication, reported a similar study, again demonstrating that in subjects with mitral stenosis and atrial fibrillation the interval between QRS of the electrocardiogram and the first heart sound varied inversely with the length of the previous cycle. This was confirmed by Wells in England.[4]

In the present study further information was sought concerning the range of the Q-first heart sound interval in mitral stenosis and in other forms of heart disease. As in the other studies, measurements were made in simultaneously recorded electrocardiograms and phonocardiograms. The Q-first heart sound interval is the time from the beginning of ventricular excitation (Q wave or onset of upstroke of R wave in limb Lead II) to the first rapid vibrations of the first heart sound. Hereinafter this interval will be referred to as the Q-1 interval. The records of subjects with a QRS duration of .11 second or more were excluded from this study because bundle branch block in itself may cause an apparent delay in the first heart sound.[5,6] Sounds associated with extrasystoles were omitted for the same reason.[7,8]

The phonocardiograms were taken either with a Cambridge Simplitrol or a Sanborn Twin Beam phonocardiograph. The paper speeds on the respective machines were 50 and 75 cm. per second, allowing one to measure with an accuracy of .01 second. The measurements by independent examiners of records were always within .01 per second. Ten intervals of each subject were measured and averaged. The subjects were resting in the supine position when the records were obtained. Phonocardiograms were routinely taken at the apex, Erb's point, the pulmonic and aortic valve areas but measurements were made from the apex recordings.

RESULTS

In a group of a hundred patients with cardiac lesions of all types except those involving the mitral valve the mean Q-1 interval was found to be .04 second with a standard deviation of .01 second. Values of normal man compiled from different sources by Wiggers[9] are in close agreement with these figures.

In a group of seventy-five subjects with mitral stenosis as the only lesion, the mean Q-1 interval was .06 second with a standard deviation of $\pm$.03 second.

These data are presented as frequency distribution graphs in Figure 1. Inspection of these charts demonstrates a wide spread of the Q-1 interval in the mitral stenosis group, ranging from .04 to .10 second. But more striking is the fact that 45 per cent of the values are .07 second or greater whereas in the group of subjects with normal mitral leaflets no intervals of .07 second or greater can be seen.

In subjects with mitral stenosis and atrial fibrillation the length of the Q-1 interval was found to vary inversely with the length of the previous diastole, as reported earlier.[2–4] No such variation was found in patients who had atrial fibrillation but who did not have mitral stenosis. In this latter group no significant

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difference was found between those subjects with sinus rhythm and those with atrial fibrillation. In the mitral group the length of the Q-1 interval paralleled the severity of the stenosis. Age seemed to be the factor determining rhythm. In this population atrial fibrillation was uncommon before the age of forty unless active carditis was present.

TABLE I

Patient	Q to First Sound (seconds)		Second Sound to Opening Snap (seconds)	
	Pre-operative	Post-operative	Pre-operative	Post-operative
1	0.10	0.07	?*	0.04
2	0.07	0.05	0.05	0.08
3	0.08	0.04	0.04	0.07
4	0.08	0.05	0.05	0.08
5	0.07	0.05	0.06	0.08
6	0.08	0.06	0.06	0.11
7	0.08	0.05	0.07	0.10
8	0.06	0.04	0.06	0.09
9	0.08	0.05	0.04	0.08
10	0.07	0.05	0.05	0.08
11	0.09	0.06	?*	0.05
12	0.06	0.04	0.06	0.10
13	0.08	0.08	0.05	0.05
14	0.07	0.05	0.06	0.09
15	0.06	0.05	0.04	0.08
16	0.07	0.04	0.08	0.12

* Opening snap not identified on phonocardiogram.

Twenty-seven persons with mitral insufficiency were also studied. A mean Q-1 interval of .04 second with a standard deviation within ±.01 second was found, a normal value in agreement with the observations of Brigden and Leatham.[10]

A delay in the interval from the beginning of the QRS to the most intense portion of the first sound was also demonstrated in mitral stenosis, in the presence of which the mean was found to be .09 second (standard deviation = ±.03) whereas in mitral insufficiency the mean was .05 (standard deviation = ±.01) and in the control group .05 (standard deviation = ±.01) second.

Although this investigation was designed primarily to show the variation in the Q-1 interval, a record was also made of the frequency of occurrence of the opening snap of the mitral valve. The opening snap of the mitral valve was the term given by Rouches[11] in 1888 to a high pitched scund of short duration which closely follows the second sound by which is of maximal loudness just inside the apex rather than at the base, as would be the case with a split second sound. This sound was recognized as early as 1835[12] as a frequent accompaniment of mitral stenosis but was labelled an apical split second sound.

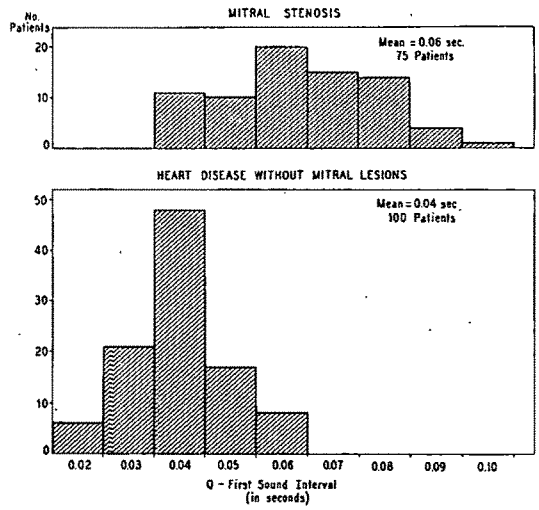


FIG. 1. Variation of the interval from the onset of electrical activation of the ventricles to the first rapid vibrations of the first sound as seen in the simultaneously recorded lead II of the electrocardiogram and the apical phonocardiogram. Subjects with QRS times 0.11 second or greater were excluded.

An opening snap was found on phonocardiography in seventy-two of the seventy-five subjects who had mitral stenosis but in only one patient who did not have significant mitral stenosis; this occurred in a patient whose condition was diagnosed as mitral stenosis but it was found at operation that only one cusp with its chordae tendineae became fibrotic. This produced a moderate insufficiency but no appreciable stenosis. A similar high incidence of the opening snap in mitral stenosis has been reported recently by Mounsey[13] who recorded it in thirty-two of thirty-three patients with mitral stenosis. This sound, it should be pointed out, is commonly confused with a gallop or a split second sound.

The presence of an opening snap is of great diagnostic significance. It is a more frequent finding in severe mitral stenosis than is a diastolic murmur. We were unable to hear or record a diastolic murmur in seventeen patients with mitral stenosis yet in fourteen of these an opening snap was recorded.

The data from preoperative and postoperative tracings in sixteen patients who underwent mitral valve fracture are presented in Table 1. In every instance except one a shortening of the Q-1 interval followed surgery. (The one patient who was the exception derived no benefit from the operation.) Also, the interval from the beginning of the second sound to the opening snap of the mitral valve lengthened with surgery. This latter interval (O-S interval) apparently varies with isometric relaxation of the left ventricle. No change in the left ventricular volume occurs during this interval but when the pressure in the left ventricle falls below that in the left atrium the mitral valve snaps down and filling of the ventricle begins. When there is no change in the systemic arterial pressure a lengthening of the isometric relaxation time (O-S interval) can be interpreted as the result of reduction of the left atrial pressure following mitral valvotomy.

DISCUSSION

The observations reported here clearly demonstrate the frequency of prolonged Q-1 intervals in mitral stenosis and the absence of such delay with cardiac lesions of other types. In any subject with a Q-1 interval of 0.07 second or greater (in the absence of bundle branch block), it may be taken for granted that there is a significant degree of mitral stenosis. This knowledge may have considerable clinical usefulness. As an example, in a patient with unexplained heart failure no murmurs were heard at first. The usual laboratory investigations such as electrocardiography and fluoroscopy revealed only atrial fibrillation and enlargement of all chambers of the heart. Mitral stenosis was strongly suspected because the Q-1 interval was 0.10 second. Later, when the heart failure was better controlled, a diastolic rumble was heard and recorded at the apex. At operation a very tight mitral valve orifice was found.

In a clinic such as ours where the end stages of disease are commonly seen, mitral stenosis without murmurs is frequently encountered. When the cardiac output falls to very low values the typical mitral rumble frequently disappears. If the mitral valve is extensively calcified the first sound often loses its usual loud snapping quality and the opening snap may be absent.[14] It is under these circumstances that the delay of the first heart sound is of real help in establishing the diagnosis; in several of these cases preoperative confirmation was provided by demonstrating calcified valves in slit kymograms.

Mid-diastolic murmurs are quite common in congenital heart disease.[15] The presence of such murmurs invariably arouses speculation concerning the presence of mitral stenosis. If no ventricular conduction defect is present and the first heart sound is delayed, it will be found that mitral stenosis also is present. Also, an Austin Flint murmur may be definitely excluded in a patient who has aortic insufficiency and a presystolic murmur if a delay in the onset of the apical first sound is found.

The mechanism by which the first sound is delayed in mitral stenosis can be explained readily by the hypothesis[16,17] that the first heart sound is caused by sudden tensing of the auriculoventricular valves and chordae tendineae when the auriculoventricular septum is pushed in the direction of the auricle. In mitral stenosis the left atrial pressure is high while the end diastolic pressure in the left ventricle is low. The mitral valve does not close until the left ventricular pressure exceeds that of the left atrium. This disparity of pressures in the atrium and ventricle is found consistently in mitral stenosis.[18,19] This is illustrated in Figure 2 which demonstrates pressures obtained in the operating room from both the left atrium and the left ventricle before and after mitral fracture.

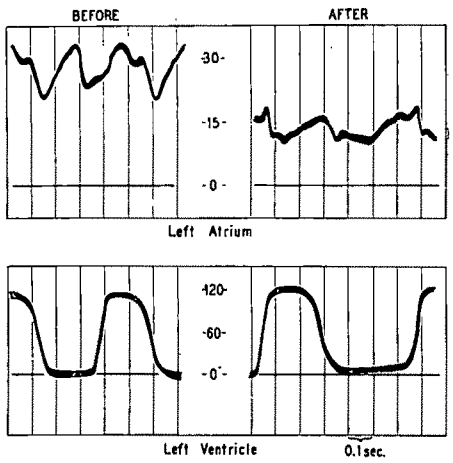
In Figure 3 are presented data from the same patient. Here ventricular pressures were recorded at a greater sensitivity of the strain gauge so that the atrial curves could be traced over the segments of the ventricular pressure curves. Also indicated in this illustration is the position in time of the first heart sound, second sound and the opening snap as recorded preoperatively and postoperatively. Although the ventricular end diastolic pressure and the electrical mechanical interval remained constant before and after surgery, the time required by the ventricle to develop pressure greater than that in the left atrium fell from 0.08 second to 0.05 second after enlargement of the mitral orifice. These time periods agree perfectly with the Q-1 intervals determined phonocardiographically pre- and postoperatively. It is also apparent that a significant degree of mitral stenosis remained after surgery, as indicated by a difference of 10 mm. Hg between the end diastolic pressures in the atrium and the ventricle. When these pressures are equal the onset of the first sound begins with ventricular contraction. In left

ventricular failure and in constrictive pericarditis left atrial pressure is also elevated but there is no impediment to ventricular filling as in mitral stenosis, and in these instances the end diastolic pressures are equal in the left atrium and ventricle. Hence no delay would be expected in the onset of the first sound and none has been observed.

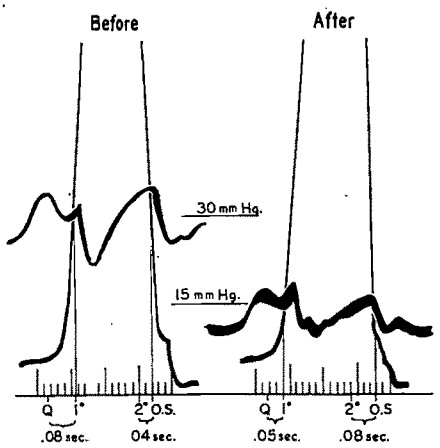
It is also of interest that in subjects with significant mitral insufficiency the Q-1 interval is normal. Apical systolic murmurs, which are not uncommon in mitral stenosis, do not necessarily indicate any serious degree of mitral insufficiency. A recent experience vividly brought this lesson home to us. A middle-aged woman who was studied in our laboratory was found to have a normal QRS time, a loud first sound, a grade IV pansystolic apical murmur and an opening snap which was recorded but not heard by most examiners. Because the first sound was loud and delayed (Q-1 $\pm$0.09 second) it was predicted that a severe degree of mitral stenosis was present and that mitral insufficiency would be minimal in spite of the intense murmur. This prediction was confirmed by postmortem examination a week later when a very rigid, tightly stenotic valve was found. The cusps of this valve could not possibly close completely during systole. However, when the mitral valves are severely incompetent, as much blood may be injected into the atrium as into the aorta. In instances such as this the first sound is faint or lost in the systolic murmur but its onset is never delayed. A gallop, which is rare in mitral stenosis even when complicated by right ventricular failure, may be the loudest sound heard in severe mitral insufficiency and has been confused with the first sound. Such gallops occur 0.10 to 0.18 second after the second sound and therefore are not easily confused with opening snaps occurring between 0.02 and 0.13 second after the second sound. For an excellent discussion of mitral insufficiency the paper of Brigden and Leatham[10] should be consulted.

A long Q-1 interval and a short O-S interval are characteristic of tight mitral stenosis. Using the surgeon's estimate of the mitral orifice at operation, Wells[4] demonstrated a better correlation of the size of the mitral orifice with a consideration of both the Q-1 and O-S intervals together than when considering either separately. By subtracting the O-S interval from the Q-1 interval values greater than minus 0.01 second were found with severe mitral stenosis. If the values gave more negative results than minus 0.015 second, an orifice greater than 1 sq. cm. could be expected.

Our experience in general confirms Wells'



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FIG. 2. Left atrial and ventricular pressures recorded by needle puncture of these chambers before and after mitral valvuloplasty.

FIG. 3. Mitral valvuloplasty. Pressures from the same patient as in Figure 2 but in this instance the ventricular pressures were recorded at a greater sensitivity of the strain gauge so that the atrial curve could be superimposed on the ventricular curve. Q refers to the onset of the QRS complex of the electrocardiogram; 1° refers to the first sound, 2° to the second sound and O.S. to the opening snap. Phonocardiograms were taken preoperatively and postoperatively (the heart rates at these times were the same). The electrical–mechanical interval of the left ventricle remained constant at 0.03 second.

views but there are rare exceptions and diagnostic pitfalls which should be briefly discussed. Figure 1 demonstrated that only 45 per cent of the subjects who had mitral stenosis had a Q-1 interval outside the range of the control group, which varied between 0.02 to 0.06 second. Presumably, most of the patients whose Q-1 interval fell within the normal range had only a moderate or minimal degree of mitral stenosis. However, in rare instances we have found that subjects with normal Q-1 intervals may have tight mitral stenosis at necropsy. The explanation for this apparent discrepancy may be found in a very short electrical-mechanical interval of the left ventricle: that is, the time between the electrical activation of the ventricle (Q wave or upstroke of R wave of the electrocardiogram) to the onset of the left ventricular pressure rise. In those persons in whom the heart does not have ventricular conduction defects this time varies between 0.02 second and 0.06 second with a mean of 0.04 second,[19] which is the mean of the Q-1 interval in the group of patients who do not have mitral stenosis.

If a patient with severe mitral stenosis had a left ventricular electrical-mechanical interval of 0.02 second, and 0.03 second further elapsed before the ventricle could develop pressure necessary to exceed the left atrial pressure so that the mitral cusps could snap shut, then the Q-1 would be the sum of these two values, or 0.05 second, which is within the normal range.

Although the electrical-mechanical intervals were determined by needle puncture of the ventricle at operation, this same information may be obtained by the simple, non-hazardous method of recording the apex impulse. The onset of the systolic deflection of the apex beat coincides perfectly with the ventricular pressure rise determined directly. This technic was utilized by Cossio[20] to support the valvular theory of the first heart sound. In patients with mitral stenosis he was able to record the usual apex motion occurring without delay, followed by a sharp apical shock synchronous with the delayed first heart sound.

Another factor affecting the Q-1 interval in mitral stenosis is the rate of rise of ventricular pressure. In twelve instances in which left ventricular pressure curves were obtained the rate of pressure rise varied between 4 mm. Hg and 14 mm. Hg per 0.01 second. Preliminary studies of our data suggest that the rate of rise is much slower in patients with extensive myocarditis, whether active or healed.

In one instance we mistakenly made the diagnosis of mitral stenosis by confusing as the first sound a loud, early systolic sound which was of maximal intensity at the pulmonic area rather than at the apex. Such sounds are usually associated with pulmonary artery dilatation and pulmonary hypertension.[21] This particular patient had a ruptured aortic cusp which produced a continuous murmur from the second sound to the "early systolic click," with no recognizable first sound. To avoid this type of error it is wise to compare the intensity of the first sound both at the apex and at the base of the heart. When a dilated aorta or, more commonly, a dilated pulmonary artery is present a sound is emitted as the semilunar valves open or shortly thereafter. Usually no sound can be detected by the phonocardiogram when the semilunar valves open; however, with dilatation of either of these major vessels the sound may be as loud as that arising from the auriculoventricular valves.

The observations reported herein provide valid evidence that ventricular muscular contraction contributes no audible element to the first sound. In extreme cases the first sound may not begin until 0.06 second after ventricular contraction occurs. It also appears that in mitral stenosis the first heart sound is caused by the tensing of the mitral valve and its chordae; the tricuspid valve contributes no easily audible portion when the delay is great, otherwise a split first sound rather than a delayed sound would be recorded. For example, in a patient with a Q-1 interval of 0.09 second the right ventricular pressure exceeded the right atrial pressure 0.04 second after the Q wave of the electrocardiogram, yet no sound could be recorded at that moment even close to the sternum. Rarely has it been possible to demonstrate by great amplification of the phonocardiograph a faint sound synchronous with closure of the tricuspid valve in instances of mitral stenosis with an intense and delayed first sound.

This situation may be similar to that in left ventricular failure with severe systemic arterial hypertension. In patients with such conditions there is often a very faint first sound but after correction of the failure and reduction in the residual volume in the left ventricle the first sound becomes more intense. Presumably, in failure with an increase in residual volume and

rapid ventricular filling the valve leaflets are approximated at the end of diastole, whereas in healthy persons they may be deep in the ventricle when systole begins and sudden tensing causes a much louder sound. This can be illustrated with a handkerchief producing a faint or loud sound depending on the slackness of the cloth before it is snapped taut.

Perhaps the tricuspid valve normally causes little sound because of physical differences from the mitral valve. In our experience with patients who have pure right ventricular failure gallop is often absent, never more than barely audible, in contrast to gallop in left heart failure which usually is easily audible. However, inaudible vibrations related to gallop phenomena are usually recorded by ballistocardiography and roentgenkymography in patients with right ventricular failure.[22] It is also of interest that Thayer,[23] in his classic paper on the third heart sound, stated that he could easily hear a third sound over the exposed left ventricle of a dog but not over the right ventricle. We have had the opportunity of studying a woman with cor pulmonale and right thoracoplasty whose heart beat directly against the subcutis to the right of the sternum. It was possible to record a presystolic gallop directly over the right atrium, although the gallop was inaudible elsewhere over areas with the usual chest structures intact. This too suggests that normally the tricuspid sound may be faint and poorly transmitted to the precordium. Rytand[24] concluded from another type of study that the first heart sound is largely of mitral rather than of tricuspid origin in normal persons and in patients without mitral stenosis.

The theory of the valvular origin of the first sound explains such common clinical phenomena as the variation in intensity of the first sound in complete heart block[24,25] with varying P-R intervals,[25,26] auricular fibrillation[27] and ventricular tachycardia.[17] The theory of the muscular origin of the first sound cannot explain an intense first sound heard with a ventricular extrasystole which fails to develop enough ventricular pressure to open the semilunar valves or a faint first sound in aortic insufficiency in which the stroke volume may be tremendous. All of these observations are explained neatly by the valvular origin of the first sound. With intense first sounds the auriculoventricular valves are deep in the ventricle and the chordae tendineae are slack when ventricular contraction begins; with faint first sounds the valves are closely approximated at this time. Mitral stenosis or the presence of a short P-R interval should be suspected, therefore, when a loud first sound is heard in a patient with congestive failure.

The lengthening of the interval from the second sound to the opening snap of the mitral valve following mitral surgery has been demonstrated. Figure 3 easily explains this phenomenon. It can be appreciated from this illustration that three variables affect the length of this interval. They are: (1) the height of pressure in the ventricle at the end of systole, (2) the speed of isometric relaxation and (3) the height of the atrial pressure. With reduction of atrial pressure after surgery and with no change in the other variables a lengthening of this interval occurs. In such instances there was no change in ventricular pressure or speed of isometric relaxation, and the O-S interval increased from 0.05 second preoperatively to 0.08 second postoperatively. These observations confirm the views of Margolies and Wolferth[28] expressed in their classic paper on the importance and the mechanism of production of the opening snap in mitral stenosis.

SUMMARY

1. The time of onset of the first heart sound is related to the simultaneous electrocardiogram in subjects with mitral stenosis, mitral insufficiency and heart disease without mitral valve disease.

2. A delay in the first heart sound occurred in patients who had mitral stenosis but who had no other lesions if ventricular conduction defects were absent. The degree of delay paralleled the severity of the mitral stenosis. This delay of the first heart sound has proved to be of diagnostic value, particularly since it is usually greatest when the typical murmurs are absent. No delays occur with significant mitral insufficiency.

3. Significant shortening of the Q-first sound interval occurs with successful enlargement of the mitral orifice.

4. The delay of the first sound can be explained by the disparity between the end diastolic pressures in the left atrium and left ventricle. The sound does not occur until ventricular pressure equals or exceeds atrial pressure.

5. Ventricular contraction does not contribute to the audible portion of the first sound.

Moreover, this sound probably arises chiefly in the mitral rather than in the tricuspid valve.

6. The length of the interval between the second sound and the opening snap of the mitral valve is, in general, inversely related to the severity of the mitral stenosis. This interval lengthens with successful mitral surgery.

7. The mechanism of production of the opening snap is discussed and observations offered which confirm the views of Margolies and Wolferth[28] concerning this phenomenon.

Acknowledgment: The author wishes to thank Dr. William Dock and Dr. D. A. Rytand for their advice and criticism.

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The Triad of Tachycardia, Digitalis Toxicity and Mercurial-fast Edema in Congestive Heart Failure Complicated by Pulmonary Embolism

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PULMONARY embolism is of frequent occurrence,[1-3] particularly in patients with congestive heart failure.[4-6] The clinical features of thromboembolic phenomena have been well defined in non-cardiac disease,[7,8] but the manner in which pulmonary embolism may disturb congestive heart failure has not been clear. Attention was called to this problem by the development, in a number of patients with pulmonary emboli, of the triad of intractable edema, disproportionate tachycardia and digitalis toxicity. This investigation was undertaken to evaluate the occurrence of this triad in patients who died of chronic congestive heart failure complicated by pulmonary embolism and to compare them with another group of patients with fatal heart failure whose course was not thus complicated.

METHODS

From January 1946 to July 1954, at the Peter Bent Brigham Hospital, forty-seven patients who had congestive heart failure died as a major consequence of multiple pulmonary emboli. The clinical records and autopsy protocols were reviewed, and special emphasis was placed upon a search for evidence of intractable edema, disproportionate tachycardia and digitalis toxicity prior to death. Three additional patients with whom the author had personal experience at the New England Center Hospital were included. The records of fifty patients from the Peter Bent Brigham Hospital who died in congestive failure not complicated by pulmonary embolism were reviewed from the same standpoint. In this group it was necessary to include five postoperative cardiac surgical patients. No patient was included who had experienced a recent myocardial infarction.

That the groups were comparable so far as types of heart disease may be seen from Table I; the etiology of the heart disease in each group was about the same, namely, half rheumatic and half coronary artery disease. Two cases of non-specific interstitial myocarditis were included in the group with pulmonary embolism. Digitalis toxicity was noted by electrocardiography by well established criteria[9] in forty patients and clinically in six more. Tachycardia was diagnosed when the apical rate was more than 110 per minute.

TABLE I

Condition	Pulmonary Embolism Group	Control Group
Coronary artery disease	28	25
Rheumatic heart disease	20	25
Interstitial non-specific myocarditis	2	..

RESULTS

Intractable Edema. Thirty-five patients in the pulmonary embolism group received mercurials as contrasted with thirty-eight in the control group. Thirty-two of the thirty-five in the pulmonary embolism group were refractory to mercurial diuretics as opposed to only ten who were refractory in the control group. (Table II.)

Therefore it seemed that refractoriness to mercurial diuretics suggested the presence of

pulmonary emboli, and a satisfactory diuresis with mercurials appeared to indicate that multiple pulmonary embolization was not present.

Digitalis Toxicity and Tachycardia. The combination of digitalis toxicity and tachycardia was present in twenty-six cases of the pulmonary embolism group and in seven cases of the control group. Thus the coincidence of tachycardia plus digitalis toxicity was well correlated with pulmonary embolization. (Table III.)

Even more convincing was the association of tachycardia, digitalis toxicity and mercurial fastness. This triad was almost invariably associated with pulmonary embolism. (Table IV.)

The electrocardiogram presented distinctive evidence of acute cor pulmonale in only six patients in the pulmonary embolism group; no such evidence was noted in the patients in the control group. In four cases in each group a left-ward shift of the transition zone was noted. It would appear that this change is not reliable evidence of acute cor pulmonale in the presence of congestive heart failure.

Five patients in the embolism group and one in the control group had frank thrombophlebitis.

TABLE II

Condition	Pulmonary Embolism Group			Control Group		
	Refractory to Mercurials	Not Refractory to Mercurials	Mercurials Not Given	Refractory to Mercurials	Not Refractory to Mercurials	Mercurials Not Given
Coronary artery disease	14	1	13	4	13	8
Rheumatic heart disease	17	2	1	6	15	4
Interstitial myocarditis	1	..	1	..	..	..
	32	3	15	10	28	12

TABLE III

Condition	Digitalis Intoxication plus Tachycardia	
	Pulmonary Embolism Group	Control Group
Coronary artery disease	10	2
Rheumatic heart disease	15	5
Myocarditis	1	..
	26	7

TABLE IV

Condition	Mercurial Fastness plus Tachycardia plus Digitalis Intoxication			
	Pulmonary Embolism Group	Mercurials Given	Control Group	Mercurials Given
Coronary artery disease	10	15	0	17
Rheumatic heart disease	9	19	2	21
Myocarditis	1	1	..	..
	20	35	2	38

COMMENTS

The inception of pulmonary embolism, particularly in established congestive heart failure, is not always easy to appreciate. Few patients have the characteristic association of pain, dyspnea and hemoptysis. The following symptoms have been described as signalling the onset of this complication; these symptoms include bouts of palpitation and arrhythmias, syncopal attacks, recurrent and particularly nocturnal episodes of dyspnea, symptoms of acute anxiety, unexplained epigastric distress and anginal syndromes.[5,7,8,10,11] The association of these symptoms with chronic congestive failure has heretofore not been emphasized.

This study calls attention to the possibility of another clue to this development, namely the triad of tachycardia, digitalis intoxication

and resistance to mercurial diuresis. Satisfactory diuresis with mercurials appears to indicate that multiple pulmonary embolization is not present.

Acknowledgment: The author is very grateful to Dr. Harold D. Levine of Boston for his criticism and advice, and to Dr. George Thorn of the Peter Bent Brigham Hospital and Dr. Robert P. McCoombs of the New England Center Hospital for permission to examine the records of these cases.

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Subcutaneous Emphysema of Gastrointestinal Origin*

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SUBCUTANEOUS emphysema is a common finding and may develop from four sources: (1) a break in the continuity of the respiratory tract:[1] (2) gas-forming infections;[2–4] (3) atmospheric or intra-abdominal air through a break in the skin, genitourinary tract or peritoneum;[5–9] (4) a rent in the gastrointestinal tract. The importance of considering the latter point of origin when making the differential diagnosis of the cause of subcutaneous emphysema merits emphasis because of the differences in treatment and prognosis.

Two cases are presented of extensive subcutaneous emphysema arising from the intra-abdominal portion of the gastrointestinal tract. A review of the literature on this subject has been made and the reported cases tabulated. A discussion of the etiology, findings, mechanism of development and means of diagnosis is presented.

HISTORICAL OBSERVATIONS

Abeille[10] in 1853 was the first to describe a case of subcutaneous emphysema arising from the gastrointestinal tract. Von Reich[11] reported a most unusual intestinal injury that was accompanied by subcutaneous emphysema in 1859. Demarquay[12] was the first to record this entity following rectal surgery in 1860. In the same year Ericksen and Tatum[13] described perineal emphysema from transrectal cystotomy. Roger[14] gave the first comprehensive review of the general subject of subcutaneous emphysema in 1862 and reported a case arising from tuberculous enteritis with perforation. He cites Bouley and Reynal who observed subcutaneous emphysema in animals from perforation of the intestinal tract due to ingested foreign bodies. Newman[15] had a patient with a ruptured stomach and subcutaneous emphysema in 1868. Lediard[16] in 1877 reported a case similar to that of Roger, of a patient who died from typhoid fever. Poensgen, Kussmaul and Von Recklinghausen[17] described a patient with perforated gastric ulcer and massive subcutaneous emphysema in 1879.

Faber,[18] Nelson,[19] Rankin and Judd,[20] and Finnegan[21] added cases to the literature between 1885 and 1922. Vigyazo[22,23] in 1926 did likewise and mentioned a number of French physicians (LaPointe, Okinczye, Dujavier, Dantin and Bonneau†) who had observed subcutaneous emphysema following appendectomies. Subsequently, a number of cases were reported.

The subject aroused a good deal of interest in the German literature in the 1920's, when the significance of subcutaneous emphysema as an almost pathognomonic sign of perforated peptic ulcer was emphasized.

At the present time a review of the literature resulted in the collection of thirty-four cases which fall into this classification, to which we wish to add two. The original reports of Kappis[24] and Kausche[25] could not be located but they are briefly cited by Vigyazo.[22,23]

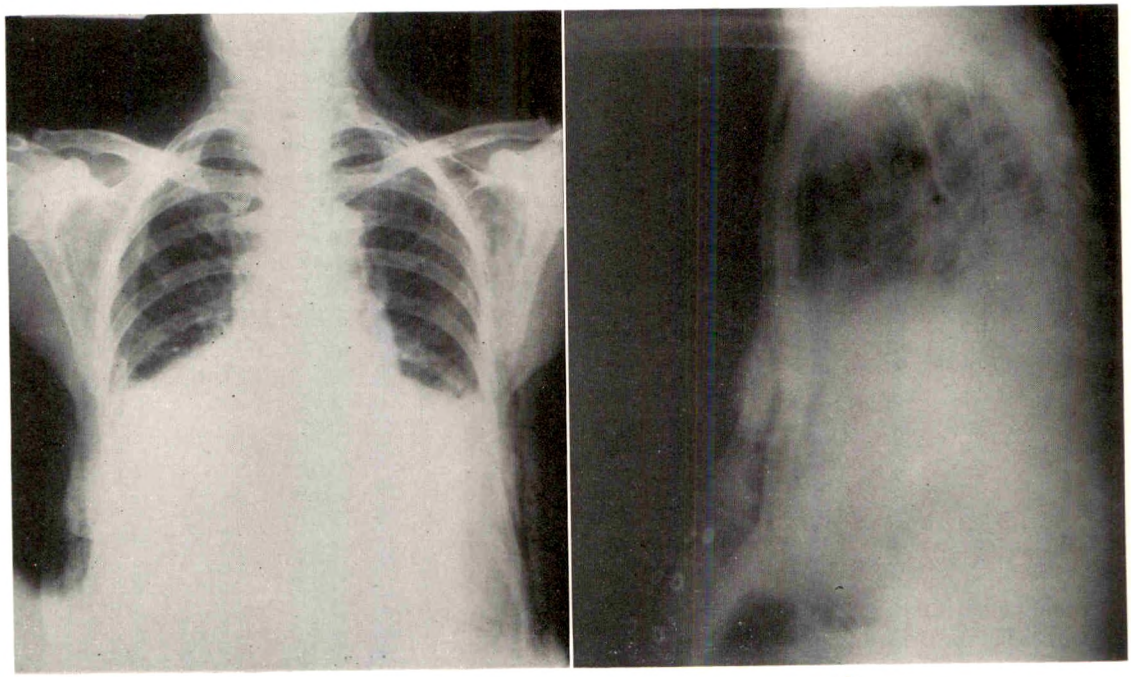
CASE REPORTS

In January, 1954, massive subcutaneous emphysema developed after gastrointestinal surgery in two patients under our care. This complication caused us considerable alarm. Neither we nor any of our colleagues had ever witnessed such an event. These cases generated an interest in the subject when the unusualness of the condition was appreciated.

CASE 1. A seventy-seven year old white housewife was admitted to the hospital with a history of bilateral, cramping, hypogastric

† Not listed in Index Medicus or Surgeon General's Report.

*From the Departments of Medicine and Surgery of the Hollywood Presbyterian Hospital, Olmsted Memorial, Los Angeles, California.



1 2

FIG. 1. Case 1. Radiograph of the chest taken the day of appearance of the subcutaneous emphysema. The widened mediastinum and partial atelectasis of the lower lobes is due to the elevation of the diaphragm from abdominal distention. Mediastinal emphysema is not present. The subcutaneous emphysema extends from the abdominal to the neck areas.

FIG. 2. Case 1. Left lateral radiograph showing the subcutaneous air over the anterior chest, the maximum amount being present over the upper abdomen.

abdominal pain, nausea, vomiting and progressive abdominal distention of five days' duration. Oophorectomy with uterine suspension and elective appendectomy had been performed fifty years before.

Physical examination revealed moderate dehydration and a distended, non-rigid, tender abdomen with absent peristalsis. Blood and urine examinations were normal. Radiographic examination of the abdomen showed classic findings of small intestinal obstruction. After Levin tube suction and intravenous fluid therapy, the abdomen was opened. Endotracheal anesthesia was used.

There were multiple fibrous adhesions from the previous surgery. The ileum was markedly distended. In its distal third several loops were plicated by adhesions that obliterated the lumen. A 2 cm. oval antemesenteric area of intramural gangrene was present at the site of obstruction. The obstructed segment was resected en bloc and a side-to-side anastomosis performed. A 1 per cent solution of neomycin was placed in the lumen of the bowel and the peritoneal cavity. The abdomen was closed in layers and three wire retention sutures placed. Drains were not inserted.

The pathologic diagnosis was acute inflammatory obstruction of the small bowel with insular gangrene of the wall.

Postoperatively the patient passed flatus and stool on the fourth day and was given full oral intake on the fifth day. She remained afebrile until a temperature of 101°F. was noted on the seventh postoperative day. Pyuria and hematuria were discovered on urinalysis at this time and antibiotic therapy was instituted.

Sometime between the evening of the eighth and the morning of the ninth postoperative day, a slightly tender, diffuse, subcutaneous crepitation developed. This extended over the entire anterior abdominal and thoracic wall from pubis to the neck and laterally over the back to the infrascapular region. There were no evidences of disruption of the pulmonary tree clinically. Roentgenographically there was neither mediastinal emphysema, pneumothorax nor pneumoperitoneum. (Figs. 1 and 2.) The ascending colon

was moderately distended. A dome-like pocket of gas was present in the superior portion of the incision. When this pocket was opened, gas with the odor of hydrogen sulfide and approximately 90 cc. of red-brown seropurulent fluid were released. Digital exploration of the wound revealed that the anterior fascial repair was intact. However, in the fascia to the left and lateral to the rectus sheath and superior to the line of suture, a 2 cm. oval defect was felt. The diagnosis of fistula of the small bowel was apparent. Aerobic cultures of the purulent fluid revealed predominantly Aerobacter aerogenes; anaerobic cultures did not yield clostridium organisms.

For the succeeding five days the patient had a low remittent fever which reached 100.6°F. There was no evidence of generalized peritonitis. Antibiotics, parenteral fluid and electrolyte therapy were continued. The subcutaneous emphysema gradually decreased. It disappeared in the areas of the neck and chest by the fifteenth postoperative day. Formed stool was passed per rectum on the seventeenth postoperative day. By the twentieth postoperative day the last remaining traces of the emphysema had disappeared both clinically and radiographically.

Phlebothrombosis of the lower extremities developed and one episode of pulmonary embolism occurred on the twenty-second postoperative day. Anticoagulant therapy was instituted with an uneventful course thereafter. The patient was discharged ambulatory on the forty-eighth postoperative day with complete closure of the fistula.

Comment. Fever on the seventh postoperative day undoubtedly was attributable to the development of the fistula and pyuria. The urine was normal, however, when the emphysema appeared two days later. The subcutaneous emphysema developed rapidly over a twelve-hour period and spread widely from its maximum point in the anterior abdominal wall to the entire trunk and supraclavicular areas. The patient's condition was largely unaltered by the emphysema. Exploration of the intact wound provided an immediate path of escape for the gas and the emphysema no longer progressed. Fever continued until the fourteenth postoperative day and was due principally to the infection at the site of the fistula, but may have been contributed to by the subcutaneous emphysema. During this time the emphysema gradually absorbed, in spite of the continuing infection at the fistula site, and resolved completely by the twentieth postoperative day.

The delay in appearance of the subcutaneous emphysema until the ninth postoperative day eliminated the possibility of a respiratory accident during the endotracheal anesthesia. The absence of physical and radiographic signs of pneumothorax, fractured ribs, blebs and mediastinal emphysema further exonerated the pulmonary tree. There was no roentgenographic evidence of retroperitoneal gas or pneumoperitoneum, and no subcutaneous tissue reaction or evidence of gas gangrene was noted. The dome-like pocket of gas at the wound site was ascribed either to the A. aerogenes infection in the area or to the gas from the fistula, or both. Although A. aerogenes can produce gas, the extent and rapidity of formation of the emphysema, the lack of subcutaneous tissue reaction, the absence of signs of toxicity or enteritis, and the subsequent course of the patient made infection with this organism as the primary cause of the emphysema highly improbable.

We believe that the mechanism of production of the subcutaneous emphysema in this patient was primarily mechanical, i.e., a small bowel fistula broke through the parietal peritoneum, musculature and fascia, and finally connected with the subcutaneous space. Then the peristaltic action of the bowel, together with changes in intra-abdominal pressure, forced diffusion of the gas subcutaneously. Secondarily, the A. aerogenes infection in the fistulous tract and wound site may have contributed to the emphysema. A flap-like valve mechanism cannot be entirely excluded as a factor. However, we believe this highly unlikely in the gradually forming fistula as such flaps occur more commonly with sudden, spontaneous or traumatic perforations of the intestine.

Case II. A sixty-six year old white housewife was admitted to the hospital with complaints that during the past year she had noted a progressive decrease in the caliber of stools, occasional diarrhea and left lower quadrant abdominal pain almost daily. Diverticulosis of the colon was known to be present for ten years. Sigmoidoscopic examinations were essentially negative. There was no melena. Radiographic studies of the colon, in comparison with those done one year previously, showed that a constricted area of the proximal sigmoid colon had become more irregular, with a narrower lumen. Carcinoma of the colon was suspected. The

patient was prepared for laparotomy and probable colon resection.

Laparotomy was performed under spinal anesthesia through a left rectus incision. A very firm mass, consisting of the sigmoid colon, left ovary and distal half of the left fallopian tube, was found to be adherent to the base of the broad ligament. Several firm nodes were palpable in the mesocolon. The mass was resected widely en bloc, including the mesocolon and peritoneum at the base of the broad ligament. End-to-end anastomosis was performed. The abdomen was closed in layers and four wire retention sutures were placed. Drains were not used.

Pathologic study revealed chronic inflammation with no evidence of malignancy.

Postoperatively the patient became uncooperative and difficult to manage. She passed flatus and stools on the third day. A selected soft diet was started on the fourth postoperative day. During this time, however, tenderness in the left lower abdominal quadrant and a remittent fever up to 100°F. was noted. A left gutter cellulitis was suspected and antibiotic therapy instituted. Rectal examination on the thirteenth postoperative day revealed a bulging, tender mass in the left lateral supralevator quadrant.

Under spinal anesthesia the rectal wall was incised. A soft hissing noise was heard of gas passing through the incision. Digital examination through the wound revealed an ill defined space retroperitoneally and above the levator ani, and a fluctuant area in the cul-de-sac. The incision was extended into the cul-de-sac and 30 cc. of purulent fluid was immediately released. A soft rubber drainage tube was placed in the abscess cavity and sutured to the rectal wall. Cultures were not taken because of gross fecal contamination.

On the evening of the day of operation the patient was restless and pulled out the rectal tube, in spite of the sutures. The temperature was 102°F. There was slight rectal bleeding throughout the first postoperative day. She complained of desire to defecate but attempts to pass even flatus were unsuccessful because of the pain and sphincter spasm. Slightly tender, diffuse subcutaneous crepitation was noted later in the day.

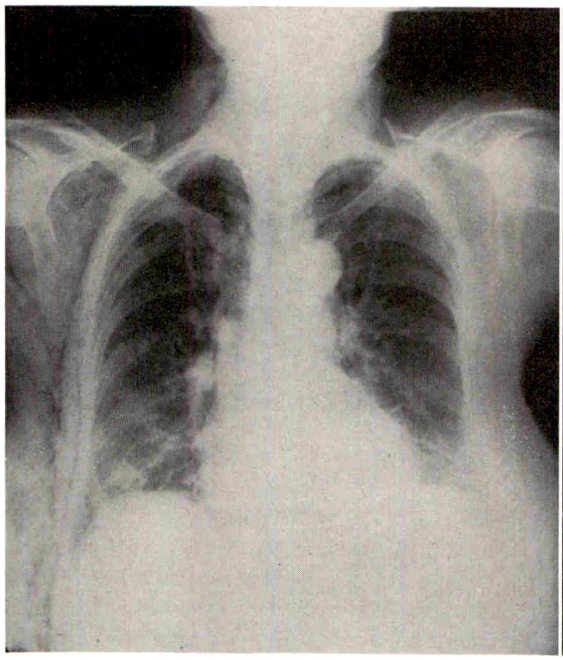
The subcutaneous emphysema extended from the lower abdominal wall to the supraclavicular areas and into the dorsal and lumbar areas of the back. Radiographs of the chest showed traces of air in the anterior mediastinum and the peritoneal cavity. (Fig. 3.) Additional roentgenograms the next day demonstrated an increase in the extent of the subcutaneous emphysema, a localized accumulation of gas in the left pararectal sulcus and retroperitoneal gas surrounding the kidneys and psoas musculature. (Fig. 4.) Antibiotic therapy was intensified. A hard rubber drainage tube was placed high into the rectum.

The patient's temperature gradually decreased to 99°F. in the next five days and remained normal thereafter. There were no complaints referable to the air beneath the skin. The subcutaneous emphysema progressively absorbed so that by the twelfth postoperative day only traces remained and by the fifteenth postoperative day it was completely gone.

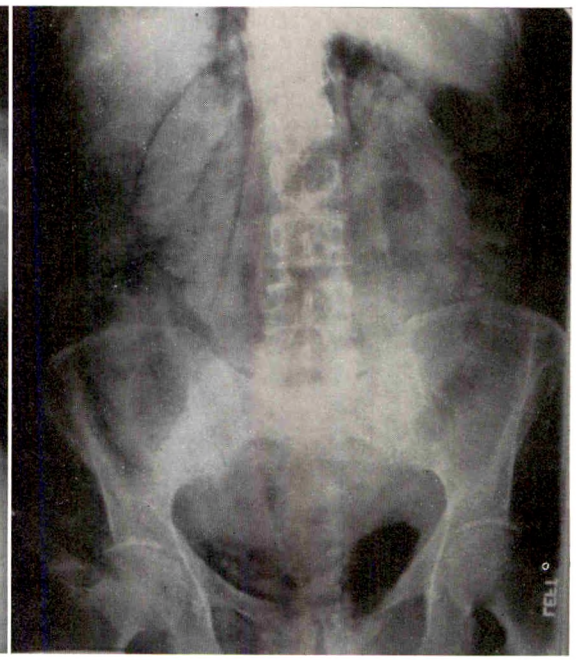
On the nineteenth postoperative day, radiopaque medium affirmed and defined the ischiorectal supralevator cavity previously visualized. The next day a radial incision was made through the left side of the anus into the inferior portion of the cavity. Purulent, foul-smelling fluid was released. Laparotomy was then performed. Exploration of the abdominal cavity revealed that the original anastomosis was intact and the mass in the cul-de-sac had disappeared. The left gutter area was well healed with no residual retroperitoneal gas. Pneumatosis intestinalis was not present. A defunctionalizing double-barrelled colostomy was constructed. The patient's course thereafter was uneventful and she became ambulatory and was discharged on the eleventh postoperative day.

Comment. The fever which was present for thirteen days prior to the first rectal surgery was due to the localizing cellulitis in the left gutter area. Following the first rectal operation the patient forceably removed the drainage tube in spite of the sutures. This further aggravated the rectal sphincter spasm and made passage of stool and flatus impossible. While the tube was out, subcutaneous emphysema developed and reached its maximum by the end of the second postoperative day. At this time a noncollapsible tube was placed high in the rectum. This provided an easy exit for the gas in the bowel. Thereafter the emphysema no longer progressed.

The temperature was increased less than one degree by these developments and decreased to normal within five days. The emphysema, of which the patient was hardly aware, gradually



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FIG. 3. Case II. Radiograph of the chest taken the day of appearance of the emphysema showing the subcutaneous gas extending from the neck to the abdominal area, principally on the right side. There are no signs of pulmonary disruption. A faint trace of air is present in the mediastinum.

FIG. 4. Case II. Flat plate of abdomen showing the retroperitoneal and perirenal gas. The gas pocket in the left pararectal sulcus is demonstrated.

absorbed so that by the fifteenth postoperative day it was no longer detectable. The pocket of gas in the left pararectal sulcus became secondarily infected from the cul-de-sac abscess and subsequently had to be drained.

The traces of gas in the anterior mediastinum were not corroborated by other physical or radiographic signs of pulmonary disruption. The presence of gas perirenally and retroperitoneally suggested that the source of the air was inferior to these areas.

The escape of gas on entering the retroperitoneal space at the time of the first rectal operation is not easily explained but may have been due to atmospheric air entrapped in the dead space produced when the left gutter was reperitonized during the initial abdominal operation. On entering the cul-de-sac purulent fluid was obtained but no escape of gas, nor were any gas bubbles mixed with the exudate. The absence of gas upon entering this abscess would seem to indicate very little if any gas formation at this site and certainly not sufficient to produce the subcutaneous emphysema. Furthermore the development of the emphysema very shortly following rectal surgery and not during the localizing phase of the infection seems more than coincidental.

We believe that the mechanism of development of the subcutaneous emphysema in this case was primarily mechanical, i.e., the operation produced a passageway from the lumen of the bowel to the retroperitoneal space. Then in the presence of a marked sphincter spasm great increases in the intraabdominal pressure from ineffectual attempts to pass flatus together with the peristaltic action of the bowel caused sufficient pressure to force the gas into the retroperitoneal area. The air then diffused both transversely to the anterior abdominal wall and superiorly through the mediastinum to the supraclavicular and neck areas. Here, too, a flap-like valve action of the rectal incision could have occurred but this would not materially alter our hypothesis.

ANALYSIS OF TABULATED CASES

Between 1853 and 1953, thirty-four cases of subcutaneous emphysema of gastrointestinal origin have been reported. Age of occurrence

TABLE I

SUMMARIZATION OF REPORTED CASES

Author	Age, Sex	Diagnosis	Operation	Course	Bacteriology	Modus Operandi
Abeille, 1853[10]	24, M	Multiple perforations of colon; etiology undetermined	None (autopsy)	Tumefaction in R.L.Q. with appearance of S.Q.* emphysema on 14th day of illness; incision with release of fetid gas and purulent exudate; emphysema spread to entire body by 15th day; died	None	None offered; 2 points of perforation in cecum with localized peritonitis and fistula formation; generalized peritonitis
Von Reich, 1859[11]	28, M	Traumatic perforation of intestine with disruption of abdominal wall musculature; skin intact	None (autopsy)	Day after injury emphysema appeared on anterior abdominal wall and spread to nipple and inguinal lines; died on 4th day after injury	None	None offered; 3 inch rent through fascia and muscle of abdominal wall; peritonitis with perforated ileum adherent to muscle wound
Demarquay, 1860[12]	?, M	Anal fistula	Incision of fistula	Emphysema developed in perineum, scrotum and lower abdomen 48 hours postoperatively; gradually absorbed; recovered	None	Intrarectal dressing caused sphincter spasm; patient's efforts to pass flatus forced gas into S.Q. spaces
Tatum, 1860[13]	72, M	Urinary retention due to urethral stricture	Cystostomy via rectum (autopsy)	Emphysema of scrotum on 1st day postoperatively with extension as high as axilla by 2nd postoperative day; died	None	None offered
Ericksen, 1860[13]	32, M	Urinary retention due to urethral stricture	Cystostomy via rectum (autopsy)	Stormy with tachycardia, chills and delirium; emphysema appeared on 5th postoperative day over posterior thighs, flanks, right shoulder and arm to wrist	None	Valvular action of rectal flap; fecal odor to gas; retroperitoneal air in pelvis
Roger, 1862[14]	12, M	Tuberculous enteritis with perforations of cecum and ileum with localized and generalized peritonitis	None (autopsy)	Tumefaction in left side of abdomen with S.Q. emphysema on 11th day of illness; spread across abdomen and superiorly to involve the entire right side of body, neck and face; on 12th day pneumonia and death	None	Adherence of ileum to anterior abdomen wall with perforation through adhesion and peritoneum and passage of gas to S.Q. spaces
Newman, 1868[15]	30, M	Ruptured stomach, pneumoperitoneum	None (autopsy)	Violent vomiting; pneumoperitoneum and S.Q. emphysema in neck and right arm 14 hours after onset; emphysema increased before death to include all of body except left arm and legs; died	None	Linear abrasions of peritoneum found at autopsy; pressure of pneumoperitoneum sufficient to cause diffusion of gas through these abrasions and then to subcutaneous areas
Lediard, 1877[16]	21, F	Typhoid fever with perforation of ileum and localized peritonitis	None (autopsy)	Stormy course; diffuse emphysema starting 9th day and advancing to 19th day—the day of death	None	Walled off accumulation of feces with erosion of the anterior abdominal wall; perforated ileum in mass; gas diffused into S.Q. spaces
Poensgen, Kussmaul and Von Recklinghausen, 1879[17]	?	Perforated ulcer of cardia of stomach	?	?	?	Spread of gas through the esophogeal hiatus into the mediastinum, the neck and adjacent subcutaneous spaces
Faber, 1885[18]	?	Perforated gastric ulcer	?	?	?	Perforation into mediastinum with spread of gas into neck, etc.
Faisst, 1920[26]	?	Anterior abdominal wall laceration	?	Emphysema of scrotum	?	Perforation of ulcer in loop of small intestine in area of abdominal wall injury

*S.Q. = subcutaneous.

TABLE I (*Continued*)

Author	Age, Sex	Diagnosis	Operation	Course	Bacteriology	Modus Operandi
Nelson, 1920[19]	54, M	Intestinal obstruction pneumoperitoneum	Laparotomy	No perforation discovered; tube drainage of pneumoperitoneum; recovered	None	None offered
Rankin and Judd, 1922[20]	46, M	Vesicocolic fistula due to perforated diverticulum of colon with obstruction of colon	Colostomy	8th postoperative day gas in scrotum; colostomy then opened; no emphysema on abdominal wall; benign course; survived	None	Secondary perforation of colon into abdominal wall with passage of gas down into scrotum
Finnegan, 1923[21]	42, M	Traumatic (pneumatic) rupture of rectum	None	No pneumoperitoneum; survived	None	Rapid spread of air up retroperitoneal spaces to neck and supraclavicular spaces
*Kausche, 1923[25]	?	Perforation of posterior wall duodenal ulcer	?	Phlegmonous inflammation with subcutaneous emphysema	None	None
*Kappis, 1924[24]	?	Postoperative perforation of bowel	?	Anterior wall emphysema	None	Pneumoperitoneum with passage of gas through traumatic holes or breaks in parietal pneumoperitoneum and then into subcutaneous spaces
Vigyazo, 1926[22]	52, M	Perforated anterior wall duodenal ulcer lesser curvature	Perforation closed	Emphysema present on admission in umbilical area; slight fever and tachycardia; resolution of emphysema in 1 day; recovered	None	(1) Gas into the subserosal area to the retroperitoneal area then to the anterior abdominal wall; (2) gas passing into leaves of hepatoduodenal ligament into the periportal tissue and then via the round ligament to the periumbilical area; (2) a projectile-like passage of gas via the route in (1)
Vigyazo, 1926[22]	?	?	Gastroenterostomy	Benign emphysema gone in a few days; recovered	?	?
Vigyazo, 1926[22]	?	Appendicitis	Appendectomy	Benign S.Q. emphysema disappeared in a few days; recovered	?	?
Podlaha, 1926[27]	73, M	Perforated gastric ulcer in cardia	Perforation closed	Benign; emphysema resolved in 5 days; recovered	None	Emphysema first appeared in supraclavicular areas; spread via esophageal hiatus and mediastinum
Korach, 1927[28]	36, M	Perforated gastric ulcer on lesser curvature	None (autopsy)	Emphysema present on admission; died	None	Emphysema periumbilical first and rapidly advanced, even after death; gas burned with blue flame and said to be hydrogen
Speed, 1931[29]	50, M	Fracture of right rami of ischium; rupture of ampulla of rectum; fracture of sacrum	Incision and drainage of ischiorectal abscess	Rapid development of scrotal and subcutaneous emphysema; emphysema gone on 25th day; recovered	None	Minute traumatic perforation of rectal ampulla with passage of gas into the scrotum and then into S.Q. spaces in area of right iliac crest and thigh
Barrington and Gardham, 1932[30]	52, M	Intestinal obstruction and emphysematous gangrene of left leg	None (autopsy)	Emphysema of left thigh from Poupart's ligament to knee and scrotum on admission; died 3 hours afterward and on 5th day of illness	Bacillus coli	Perforation of descending colon into retroperitoneal area; gaseous necrosis of muscles of thigh, iliacus and psoas
Gruca, 1933[31]	?	Acute phlegmonous appendicitis	Appendectomy	R.L.Q. emphysema present on admission	?	?

* Original reference not available to us. Cited by Vigyazo.[22,23]

TABLE 1 (*Continued*)

Author	Age, Sex	Diagnosis	Operation	Course	Bacteriology	Modus Operandi
Baumecker, 1941[33]	67, M	Carcinoma of left colon	Resection with side-to-side anastomosis	Benign course; 2nd postoperative day emphysema in S.Q. tissues; fistula then formed and spontaneously healed; emphysema absorbed in 3 weeks; recovered	None	Perforation of bowel; gas to wound site via fistula and then to S.Q. spaces
Hasselbach, 1941[34]	67, M	Inoperable carcinoma of sigmoid colon	Exteriorization of colon with cautery, perforation of proximal loop	S.Q. emphysema in 6 hours after cauterization; afebrile; emphysema gone by 17th postoperative day; survived	None	(1) Suture perforation with leak into S.Q. space; (2) deep accidental cautery perforation with S.Q. leak; (3) too firm a dressing over exteriorized loop causing gas to go into S.Q. spaces
Brown and Hinton, 1946[35]	47, M	Papillary cystadenocarcinoma of sigmoid colon	Resection of colon; reperitonealization of left lumbar gutter not possible	Febrile with peritonitis; S.Q. emphysema on 11th postoperative day; pneumoperitoneum on 21st postoperative day; emphysema gone on 24th postoperative day; recovered	B. coli communior; non-hemolytic streptococcus	Infection due to leakage of anastomosis with gas production and finally fistula formation
Dawson and Hardy, 1948[36]	73, F	Diverticulitis with emphysema and cellulitis	Excision of neurofibroma; incision of subcutaneous abscess; colostomy (autopsy)	Developed left inguinal abscess with crepitant cellulitis in thigh and perineum; afebrile; frothy, foul pus; fecal fistula developed; partial obstruction of colon—colostomy; general peritonitis	B. coli; Streptococcus fecalis	Perforation of diverticulum into pelvic retroperitoneal area; secondary superficial cellulitis followed by fistula formation
McCorkle and Stevenson, 1937[32]	62, M	Perforated anterior wall duodenal ulcer	Closure of perforation	Pneumoperitoneum and emphysema of mediastinum, neck and chest wall postoperatively; peritonitis; died 8th postoperative day	None	Pneumoperitoneum with spread via the mediastinum to the neck and thorax; no retroperitoneal air or gas in ligamentous tissue noted at surgery
Burt, 1949[37]	38, F	Foreign body giant cell granuloma of rectum, due to injected lipoidal substance	Local resection	Fever, abdominal distention; stool per rectum; S.Q. emphysema on 3rd postoperative day; laparotomy; gas in subperitoneal tissues and mesentery of colon; S.Q. emphysema gone 6th postoperative day; recovered	B. coli	Diffusion of gas from intestinal lumen along rectovaginal or retrorectal tissues to mesenteries; then diffusion through thinned-out portions of fascia to S.Q. position
Hammesfahr, 1949[6]	?	Rectal tear due to trauma	Closure of perforation	Localized pocket of emphysema in gluteal muscle; benign; recovered	Sterile	None
Hachmeister, 1951[38]	59, M	Appendicitis; Meckel's diverticulum	Appendectomy; resection of Meckel's diverticulum	Uneventful emphysema gone in 2 days; recovered	None	Leakage of suture with resulting pneumoperitoneum with diffusion into S.Q. tissues
Heinrichs, 1951[39]	50, F	Inflammatory tumor of pelvis with stenosis of sigmoid colon	Drainage; resection of sigmoid; end-to-end anastomosis and right-sided colostomy	24-hour pain in chest and cyanosis; no chest findings; 48-hours later emphysema in neck and thorax (3rd postoperative day); emphysema absorbed in about 14 days; recovered	No anaerobic growth; B. coli enterococcus	Leak of suture line with passage of gas into retroperitoneal area at site of anastomosis and then up mediastinum into neck
Borgström, 1953[40]	66, F	Fecal polyps and traumatic perforation	Diagnostic biopsy via proctoscope; closure of perforation	Rapid onset of emphysema; fever; abdominal distention; emphysema gradually disappeared; recovered	B. coli	Entrance of air into the retroperitoneal space and subsequent spread via mediastinum to the neck area and over thorax

ranged from twelve years to seventy-three years. Well over one-half of the patients were males. The exact rate of incidence is unknown. (Table I.)

The etiologic diagnoses were diverse but in all instances the intestinal tract was perforated,

TABLE II
ETIOLOGIC DIAGNOSES

	No. of Cases	Per cent of Total
Perforated peptic ulcer	7	20.6
Trauma	7	20.6
Enteritis	2	5.8
Intestinal obstruction	3	8.8
Diverticulitis	2	5.8
Appendicitis	3	8.8
Carcinoma	3	8.8
Anal fistula	1	2.9
Miscellaneous and unknown	6	17.6

although Nelson was unable to determine the site of the perforation. The principal diagnoses were perforated peptic ulcer in seven cases (20.6 per cent); traumatic perforation in seven cases (20.6 per cent); appendicitis in three cases (8.8 per cent); carcinoma and intestinal obstruction, each three cases (8.8 per cent); diverticulitis and bacterial enteritis in two cases each (5.8 per cent); one instance of anal fistula (2.9 per cent) and miscellaneous diagnoses six cases (17.6 per cent). (Table II.)

The large intestine was the most frequent site of perforation, with nineteen cases (55.9 per cent) falling into this category. The stomach was involved in six cases (17.6 per cent); the duodenum in three (8.8 per cent); the jejunum or ileum in four (11.6 per cent); the appendix in three (8.8 per cent); the colon in eight (23.2 per cent); the rectum or anus in eight (23.8 per cent), and unknown sites in two cases (5.8 per cent). (Table III.)

The correlation between the site of first appearance of the subcutaneous emphysema or the area of maximum involvement and the site of perforation in the intestinal tract is noteworthy. Emphysema of the anterior abdominal wall was associated with perforation of the small intestine in 4 cases, appendix in 3 and colon in 5. Emphysema of the scrotum, perineum or thigh was usually associated with tears in the anorectal area or colon.[5] Supraclavicular emphysema was most common with perforated gastric ulcers. (Table IV.)

In nineteen cases (55.9 per cent), the subcutaneous emphysema occurred before surgery and provided an additional sign for diagnostic evaluation. Fifteen (44.1 per cent) of the cases

TABLE III
ANATOMIC SITE OF PERFORATION

	No. of Cases	Per cent of Total
Stomach	6	17.6
Duodenum	3	8.8
Jejunum and ileum	4	11.6
Appendix	3	8.8
Colon	8	23.2
Rectum and anus	8	23.2
Unknown	2	5.8

TABLE IV
RELATIONSHIP OF AREA OF FIRST APPEARANCE OF EMPHYSEMA TO SITE OF PERFORATION

Organ	Anterior Abdominal Wall	Perineum, Scrotum or Thigh	Supra-clavicular or Neck Area	Other Areas
Stomach	2	0	4	0
Duodenum	1	0	1	1
Ileum or jejunum	4	1	0	0
Appendix	3	0	0	0
Colon	4	3	1	0
Rectum or anus	0	4	2	2
Unknown	0	0	0	1

developed postoperatively. The most common preoperative causes were perforated peptic ulcers, seven cases (20.6 per cent); traumatic perforation, five cases (14.7 per cent) and appendicitis, three cases (8.8 per cent). After surgery the subcutaneous emphysema occurred most often as a direct result of the operation or following leakage of suture lines, fistulas or peritonitis. (Table V.)

Infection preceded the occurrence of subcutaneous emphysema in sixteen cases (26.4 per cent). There was no apparent infection before the development of the emphysema in eighteen patients (53.0 per cent). In all of these latter patients emphysema developed within forty-eight hours after the onset of their illness or operation. Twelve of the sixteen cases with infection required from forty-eight hours to fourteen days for the emphysema to appear. (Table V.)

In none of the cases, with the exception of

Kausche,[25] Dawson[36] and Hardy and possibly Abeille,[10] was mention made of skin or subcutaneous tissue reaction. The cultural results are tabulated. There were no unusual organisms found—only those one might expect in any gastrointestinal tract. No one reported isolation of members of the clostridium group.

TABLE V
RELATIONSHIP OF EMPHYSEMA TO ONSET OF ILLNESS OR SURGERY AND INFECTION

	No. of Cases	Per cent of Total
Emphysema before surgery	19	55.9
Emphysema after surgery	15	44.1
Pre-existent infection before emphysema	16	26.4
No infection before emphysema	18	53.0
Emphysema within 48 hours of surgery or illness	22	64.7
With infection	4	11.6
Without infection	18	53.0
Emphysema after 48 hours of surgery or illness	12	35.3
With infection	12	35.3
Without infection	0	0.0

The extent of subcutaneous emphysema ranged from a localized process a few centimeters in diameter to massive involvement of the trunk, neck and face. The degree of emphysema bore no apparent relation to the diagnosis. In most instances the emphysema was not massive. The period of resolution of the emphysema was roughly dependent on its maximum extent and complete absorption took from twenty-four hours to three weeks. Sequelae attributable to the emphysema were not reported.

The course of the patients was quite variable. The subcutaneous emphysema itself produced no set pattern of clinical response. Many of the patients had a slight elevation of temperature and an increase in pulse rate for a day or two. The course and prognosis was more dependent on the basic nature of the disease or postoperative complication.

Seventeen (50.0 per cent) of the reported cases survived and eleven (32.4 per cent) of the patients died. In six cases the outcome is unknown but probably one of these patients also survived.[31] In only one of the nine cases reported since 1938 did the patient succumb.

The explanations of the mechanisms as offered by the various authors are briefly listed. A more detailed discussion of the mechanism of development of subcutaneous emphysema derived from an analysis of the reported cases, our experience with two patients and more recent physiologic considerations follows.

Cases of subcutaneous emphysema due to postlaparotomy pneumoperitoneum have been reported by Baumecker[7] and others[6,23,24,41] but are not truly of gastrointestinal origin and are, therefore, not included here.

POSTULATED MECHANISM

The pathogenesis of subcutaneous emphysema of gastrointestinal origin is dependent on three basic factors: perforation of the bowel; an adequate pressure gradient between the lumen of the bowel and ultimately the subcutaneous space; and the anatomic site of perforation. Infection, if present, may be an additional factor.

Perforation of the intestinal wall may occur after peptic ulceration, infection, chemical, mechanical, irradiative, pneumatic and surgical trauma and neoplasia. These are selfexplanatory and need no elaboration. Partial perforation with subsequent intestinal pneumatosis is not discussed here.[42]

Accurate pressure recordings in the lumen of the intestine and the adjacent abdominal cavity have revealed that peristaltic movements produce large pressure gradients between these areas. Such gradients are sufficient to perforate weakened portions of the wall and discharge intestinal contents into the abdominal cavity or retroperitoneal space. Expulsion of intestinal content at pressures exceeding atmospheric is possible by the contractions of the intestine alone, as evidenced by the regurgitation of gastric fluids or the involuntary passage of flatus or feces. The intra-abdominal pressure may be greatly increased by the contraction of the diaphragmatic and abdominal muscles. This increased pressure is transmitted to the contents of the intestinal tract and promotes their evacuation, especially at sites of perforation when the sphincters are intact.[43,44]

Resistance of the tissues to diffusion of gas varies with their density. Solid parenchymatous organs and serous membranes have a relatively great resistance to the diffusion of air, whereas loose areolar and fascial structures readily allow the passage of gas. If the latter types of tissue are adjacent to a perforation in the intestinal tract, a relatively great pressure dif-

ferential is established and emphysema may develop. In postoperative cases the pressure gradient may be enhanced by the presence of "dead spaces." In addition movement of the tissues in the wound site and in proximity to an intestinal perforation may increase the pressure differential and promote development of subcutaneous emphysema.[2]

The anatomic site of perforation largely determines the route of escape of the gas to the subcutaneous position. Gastric ulcers, particularly in the cardia, may perforate directly into one of the diaphragmatic hiatuses, tissues contiguous with them, or the retroperitoneal space. This would allow diffusion of the gas via the mediatinum to the supraclavicular and upper anterior chest areas. With partial perforations gas may pass from a subserous position to the aforementioned structures. Podlaha[27] placed hydrogen peroxide beneath the serosa of the cardia of the stomach and observed the gas to pass via the paraesophageal ligaments to the aortic notch and then into the mediastinum and supraclavicular area.

Subcutaneous emphysema due to perforations of anterior wall duodenal ulcers may develop by several routes. Podlaha[27] showed by the injection of hydrogen peroxide into the subserosa in the area of the pylorus that the gas bubbles passed to the hepatoduodenal and round ligaments and then to the periumbilical subcutaneous position. As proposed by Vigyazo,[22,23] a flap-like valve perforation could allow gas in the subserosal area to move posteriorly around to a retroperitoneal position, and from there to subcutaneous spaces of the anterior abdominal wall. A very sudden projectile force, and without a valve action, could produce emphysema by the same route. Posterior duodenal ulcers which have perforated allow the gas to enter the retroperitoneal position and then diffuse transversely to the anterior abdominal wall (the patient is usually lying supine in bed) or via the mediastinum to the areas at the base of the neck.

It has been observed that when the small or large intestine has been perforated the emphysema most often appears first on the anterior abdominal wall and is usually maximum close to the site of perforation. This is certainly true in acute appendicitis, and perforations of the colon ordinarily follow this same pattern. However, as in the cases of Rankin and Judd,[20] and others,[30,36] the emphysema may first become apparent in a distant area, e.g., scrotum or thigh. The usual route of exit of the gas from the bowel is directly through a pathologic defect in the parietal peritoneum or tissues contiguous with this defect into the intermuscular planes and subcutaneous spaces. Rarely, the gas may pass from the subserous position between the leaves of the mesentery to the root of the mesentery retroperitoneally and then to the subcutaneous position.[37,45]

When the perforation occurs in the anorectal area, the gas passes either to the anterolateral retroperitoneal space or to the retrorectal area. From these positions it may pass via the intermuscular planes to the subcutaneous areas of the perineum, scrotum, buttocks or lower abdominal wall; or superiorly through the paravertebral retroperitoneal tissues into the mediastinum and then into the supraclavicular and neck areas. Defects above the line of the pelvic reflection of the peritoneum may in addition produce pneumoperitoneum.

The development of subcutaneous emphysema associated with pneumoperitoneum is difficult to comprehend.[19] In postlaparotomy cases the emphysema has been explained by Baumecker[7] and others[6,8,44,46] as due to diffusion of the air through the suture holes, inadvertent tears in the parietal peritoneum and/or slow healing of the peritoneum. McCorkle and Stevenson[32] proposed in their case that the gas in the abdominal cavity traversed into the mediastinum but they found no gas in the ligamentous structures. With sufficient gas pressure in the abdominal cavity it would be anatomically possible for the gas to escape through the loose areolar tissue in the paraesophageal area into the mediastinum. As has been shown in dogs, a thinned-out parietal peritoneum at the internal inguinal ring or in a hernial sac, if present, could be other possible routes.[47] It has been ascertained that therapeutic as well as postoperative pneumoperitoneum is rarely complicated by subcutaneous emphysema.[48] Its occurrence must depend on some area in the parietal peritoneum being unusually thin or weak and easily, perhaps minutely, ruptured by the pressure of the gas in the abdominal cavity.

Infection plays a part in the development of subcutaneous emphysema in two ways. First, infection may cause perforation of the bowel, as in typhoid fever, and lead to local or generalized peritonitis with fistula formation. It may complicate any intestinal surgical procedure

with similar results. Second, certain bacilli may be gas-forming and contribute in varying degrees to production of the emphysema.

Most of the enteric organisms and also anaerobic staphylococci have the ability to produce gas in the presence of glucose. Exceptions are Salmonella typhosa, the shigella group and Pseudomonas aeruginosa together with staphylococci and streptococci within the intestinal tract, which do not produce gas with the common sugars.[49] The latter, therefore, need not be considered as a direct cause of subcutaneous emphysema.

The saprophytic clostridial organisms are capable of maintaining themselves in the intestinal tract of man. Some authorities indicate that Clostridium perfringens (welchii) is invariably present in normal human feces.[50] The production of fibrinolysins, hyaluronidase, collagenase and the toxic enzyme lecithinase by these bacilli promotes the passage of gas through the fragmenting connective tissue surrounding the muscle bundles and in the subcutaneous space and produces the entity of gas gangrene. Its occurrence has been reported after gastrointestinal and other surgery, but this is rare. It is much more commonly associated with trauma when the almost essential factors of shattered tissue and contamination with soil or other foreign material is present.[49]

Certain clinical observations seem to relegate infections with gas-forming organisms to no more than a contributory role in the production of subcutaneous emphysema. Frequently the amazing speed with which emphysema occurs and the volume of gas necessary for its widespread distribution is much beyond the biologic capabilities of such organisms. Also, the gradual resolution of the emphysema in the presence of continuing infection, its occurrence only after a disruption of the bowel, the benign nature of the emphysema, and its progression despite the susceptibility of many of these organisms to intensive antibiotic therapy would all seem to support the hypothesis that infection is not the principal cause of subcutaneous emphysema. The primary requirement is the presence of an adequate pressure gradient between the lumen of the bowel and tissues adjacent to the perforation capable of transmitting air.

Perforation of the intestinal tract occurs commonly. Why then is the complication of subcutaneous emphysema so rare? Several factors may be the explanation. The site of perforation may not be contiguous to loose areolar tissue or adhesions, or ligaments capable of transmitting the gas. Apparently in most cases the gradient between the intraluminal gas pressure and the tissue resistance to the spread of the gas is insufficient to allow emphysema to develop. The resistance of the sphincters at the normal routes of escape may be less than that of the tissues at the potential site of emphysema formation. Rapid healing of the site of perforation may prevent passage of sufficient gas to produce clinically detectable emphysema. Also, in the case of fistula formation the skin breakdown may be rapid and thereby allow a direct and easy exit for the gas. Certain therapeutic measures, such as rectal and gastric tubes and prompt opening of wound infections, facilitate passage of gas through these routes rather than into the tissues surrounding the perforation. Use of antibiotics postoperatively tends to prevent and eliminate infection which would cause the complications necessary to allow the development of subcutaneous emphysema.

DIAGNOSIS

The diagnosis of the cause and source of subcutaneous emphysema may be established from the history of the patient, nature of the surgery or injury, if any, characteristics of the emphysema, abdominal findings and elimination of the respiratory tree as the site of the disturbance.

In questioning the patient particular reference must be made to those conditions leading to perforation, among which peptic ulcer, appendicitis, diverticulitis, ingestion of foreign bodies and bacterial enteritis are the most common. As a postoperative complication subcutaneous emphysema is most likely to occur after operation or injuries which perforate or interrupt the continuity of the bowel. If infection is present at the time of surgery, disruption of suture lines and fistula formation must be considered as further instigating complications.

The rate of development of emphysema gives one a clue as to the pathogenesis of the perforation. A rapid diffusion of subcutaneous emphysema over a period of minutes to forty-eight hours after operation or the onset of the illness indicates a spontaneous or mechanical perforation of the bowel as the cause. Subcutaneous emphysema developing from forty-eight hours to days after the beginning of the illness or postoperatively is most likely due to an under-

lying infection which has caused perforation with peritonitis or fistula formation.

The first site of appearance or area of maximum development of subcutaneous emphysema suggests the approximate anatomic level of perforation of the bowel. Localized periumbilical emphysema is almost pathognomonic of perforation of an ulcer at or near the pylorus. Supraclavicular and neck emphysema with or without more extensive involvement of the trunk points first to a tear in the rectal area and secondly the perforation of a posterior duodenal ulcer. Gastric ulcer with perforation in approximation to one of the diaphragmatic hiatuses also must be considered in this situation but is rare and comprises only 1 to 2 per cent of all perforated ulcers. Localized or subcutaneous emphysema maximal on the anterior abdominal wall, in the absence of wound infection, indicates the small intestine or colon as the site of the defect. When the emphysema is greatest in the perineum, gluteal area, scrotum, thigh or lower abdominal wall, a tear in the anus or rectum is most likely.

Perforation of the intestinal tract within the peritoneal cavity gives rise to abdominal findings of localized or generalized peritonitis, pneumoperitoneum, tumefaction or fistula formation. These signs are usually absent when the rectum or anus is perforated and may be so with posterior duodenal or gastric ulcers. Roentgenographic examination of the abdomen is invaluable in determining the presence of retroperitoneal gas. The use of the proctoscope will define the exact site of the perforation in the lower bowel.

It has been observed repeatedly in patients and proved in dogs that air beneath the skin is itself a benign condition.[47] McNab[52] reported a child who lived ten years with generalized subcutaneous emphysema. Ordinary subcutaneous emphysema causes practically no tissue reaction. One may expect the patient to show a slight increase in temperature and pulse rate with the development of subcutaneous emphysema. More than this must be explained by other pathologic processes.

The rare possibility of gas gangrene must be considered. Its diagnosis rests primarily on clinical grounds. Black, friable, necrotic tissue with a surrounding zone of erythema, a foul discharge containing bubbles of gas, signs of increasing toxicity, progressive advance of the emphysema and radiographic demonstration of gas bubbles in the muscle bundles are the chief diagnostic characteristics of gas gangrene. The mere presence of Cl. perfringens, Clostridium novyi or other members of the group, as demonstrated on stained smears or culture, does not necessarily lead to and therefore indicate the existence of gas gangrene.[49]

In any event the respiratory trees must be freed of suspicion as the originating site of the emphysema.[53] The patient must be questioned as to causes of sudden increases in intrapulmonic pressure such as vomiting, straining at stool, cough and thoracic injury. The presence or absence of an infection in the respiratory tree must be determined. A search for ulceration of the oropharynx, trachea or esophagus should be made, particularly postoperatively when endotracheal anesthesia has been used. The presence of physical and radiographic evidence of atelectasis, pneumothorax, mediastinal widening or thoracic injury usually indicates that the emphysema is arising from a break in the pulmonary tract.

Subcutaneous emphysema of respiratory origin is most often limited to the face, neck, supraclavicular and upper thoracic areas. Emphysema in other areas makes the gastrointestinal tract a more likely source. In some cases there may be evidence of mediastinal emphysema clinically or radiographically which would tend to confuse the issue. However, in these situations the history, absence of other signs of respiratory disease, presence of abdominal signs compatible with perforated viscus, and proctoscopic examination will give one the correct diagnosis of the site of origin of the subcutaneous emphysema.

TREATMENT AND PROGNOSIS

Subcutaneous emphysema of gastrointestinal origin requires little or no treatment. The patients have practically no subjective complaints referable to the emphysema. If the emphysema should increase progressively and cause constrictive symptoms and findings in the neck or thorax, tension pneumothorax must again be ruled out. If treatment of the emphysema is deemed necessary, mild sedation and prophylactic gas bacillus antitoxin may be used. Multiple incisions or punctures of the skin were abandoned long ago. Therapy must be directed primarily toward the cause of the emphysema within the abdominal cavity as the prognosis of

the patient will depend on the skillful management of the perforated viscus.

SUMMARY AND CONCLUSIONS

1. A review of the literature on subcutaneous emphysema of gastrointestinal origin is presented, together with a tabulation of thirty-four previously reported cases.

2. Two additional cases are described. In one instance the subcutaneous emphysema developed from a small bowel fistula; in the other, rectal surgery had been performed.

3. An analysis of the cases in the literature is made, indicating the relationship of age, sex, diagnosis, areas of emphysema, sites of perforation, course of the patient and bacteriology.

4. The occurrence of subcutaneous emphysema arising from the gastrointestinal tract is most unusual. Its exact rate of incidence is unknown.

5. The physiologic considerations, anatomic routes of escape of the gas to the subcutaneous position and role of infection are discussed.

6. The presence of subcutaneous emphysema as an aid in the diagnosis of perforation of various levels of the gastrointestinal tract and its significance as a pathognomonic sign in perforated peptic ulcers are defined.

7. The benignity of subcutaneous emphysema and dependency of the prognosis on the basic underlying condition are indicated.

8. The treatment of subcutaneous emphysema of gastrointestinal origin is expectant and symptomatic. Therapy must be directed primarily toward the more serious intraabdominal perforation.

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Association of Antibody-coated Red Blood Cells with Ulcerative Colitis*

Report of Four Cases

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THE anemia frequently associated with ulcerative colitis is ordinarily attributed to chronic blood loss and diminished erythropoiesis secondary to chronic infection.[1a] There is the further possibility that an additional autoimmune hemolytic component occurs in some patients. We have recently had the opportunity of observing four patients with ulcerative colitis in whom a positive direct Coombs reaction[1b] was in fact, demonstrated. This article is a report of these cases, with a discussion of the possible relationship between the antibody-coated red cells and the intestinal disorder.

CASE REPORTS

CASE 1. Mrs. L. S. (No. 19691), a twenty-eight year old Negro, was admitted to The Mount Sinai Hospital December 11, 1953. The patient had been in good health until one year prior when frequent bloody bowel movements developed. Five months later because of an exacerbation of symptoms, associated with marked anemia, she received four blood transfusions at another hospital. Six weeks before admission a severe relapse occurred and three days prior to admission nausea and vomiting developed.

On physical examination the patient appeared pale and chronically ill. The findings were otherwise within normal limits. No icterus was present.

Blood examination revealed a hemoglobin of 8.2 gm. per cent; red cells, 2.94 million per cu. mm.; hematocrit, 26 per cent; red cell indices, M.C.V. 89, M.C.H. 28, M.C.H.C. 31; white cells, 18,300 with 73 per cent neutrophils, 6 per cent band forms, 17 per cent lymphocytes and 4 per cent eosinophils; platelets, 396,000 per cu. mm.; reticulocytes, 10.4 per cent. Slight anisocytosis and hypochromia of the erythrocytes were present. There were no spherocytes and the red cell fragility values were normal. Sedimentation rate was 138 mm. in the first hour. Sickling, not present on direct smear, was demonstrated using sodium metabisulfite. The serum iron level was 55 gamma per cent, with a total iron-binding capacity of 175 gamma per cent. No abnormal pigments were present in the plasma. The serum bilirubin determinations were normal. The Frei and Kahn tests were negative, as were agglutinin studies for enteric pathogens. The patient was group O, Rh negative (cde/cde).

The bone marrow aspirate was hypercellular with slight normoblastic hyperplasia. The myeloid: erythroid ratio was 1.8:1.0.

The direct Coombs reaction was 3+.† The serum contained anti-D and anti-E presumably due to isoimmunization by previous transfusions, and also a panantibody. In addition, another antibody identified as anti-e was demonstrated. This represented a specific autoantibody[2] as the patient was cde/cde. The cold agglutinin titer was 1:4.

The stool was grossly bloody. No ova or parasites were present. Urinalysis was essentially negative.

Sigmoidoscopy revealed a granular mucosa with a hemorrhagic, purulent exudate. Narrowing and pseudopolyp formation of the distal transverse and the descending colon were demonstrated by barium enema.

Treatment consisted of antibiotics, followed by five weeks of cortisone and then ACTH intravenously. On hormonal therapy gross blood disappeared from the stools and the guaiac reaction decreased in intensity to 1+, the hemoglobin level remained constant at 7.5 to 8.5 gm. per cent without transfusions, and the reticulocyte level decreased to 1 to 2 per cent. However, due to clinical deterioration ileostomy and subtotal colectomy were performed following three transfusions which elevated the hemoglobin to 11.9 gm. per cent. The subsequent course was uneventful.

The patient has done well since her discharge from the hospital. The ileostomy functions properly and the drainage contains no occult blood; the ileostomy fecal urobilinogen value is 2 mg. daily. Her most

† Positive direct Coombs reactions are graded in intensity from ± to 4+.

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recent blood count (August 1954) was: hemoglobin, 12.1 gm. per cent; red cells, 4.39 million per cu. mm.; hematocrit, 38 per cent; red cell indices, M.C.V. 87, M.C.H. 27, M.C.H.C. 32; reticulocytes, 0.8 per cent; platelets, 240,000 per cu. mm.; white cells, 9,000 per cu. mm. with 44 per cent neutrophils, 3 per cent band forms, 41 per cent lymphocytes, 5 per cent monocytes and 7 per cent eosinophils. The direct Coombs reaction was weaker than noted previously. The antibodies in the serum were unchanged.

Comment. Active intestinal bleeding was present when the positive direct Coombs reaction was first noted, making it difficult to assess the respective roles of these two factors in production of the anemia and reticulocytosis. Due to severe bloody diarrhea and the small amount of feces in the rectal discharges, it was not feasible to determine the fecal urobilinogen prior to ileostomy. Active blood loss precluded a cell survival study. Therefore, reliance was placed on less definitive laboratory studies in attempting to decide whether significant hemolysis was occurring. The hypochromia and low serum iron level reflected the chronic blood loss and, to some extent perhaps, the chronic infection. Absence of methemalbumin, the normal serum bilirubin, low serum iron and absence of spherocytes argued somewhat against active hemolysis. The fall in reticulocytes from 10 per cent to 1 to 2 per cent on hormone therapy was also accompanied by a decrease in the occult blood in the stool from 4+ to 1+. Thus it was difficult to decide whether maintenance of the hemoglobin level was due to an effect by the hormones on a hemolytic process or on the colitis resulting in decreased blood loss. Postoperatively, with no specific therapy, and with a reticulocyte level of 1 to 2 per cent, the patient has had no drop in hemoglobin. If hemolysis had been active at that low reticulocyte level, one would have expected a marked fall in hemoglobin. Therefore, although the patient's red blood cells gave, and continue to give, a positive direct Coombs reaction, the hemolytic process probably did not contribute significantly to the anemia.

Case II. Mrs. L. P. (No. 7148), a forty-four year old housewife, was first admitted to The Mount Sinai Hospital in March, 1953, with a ten-month history of abdominal cramps, bloody diarrhea and a weight loss of thirty pounds. Physical examination was unremarkable. The diagnosis of ulcerative colitis was established by barium enema studies in conjunction with the clinical and laboratory findings, which included a hypochromic anemia and 1.2 per cent reticulocytes. The patient improved on antibiotics, ACTH and transfusions, and was discharged after a four-month hospital stay. At the time of discharge the stools were no longer grossly bloody but gave a 4+ guaiac reaction. During the following six months the colitis was in remission. The patient was rehospitalized in December, 1953, because of pain in the right upper abdominal quadrant, accompanied by emesis of bile-stained material and frequent watery, bloody, bowel movements.

On physical examination she appeared chronically ill. There was no icterus. A poorly defined tender mass in the right upper abdominal quadrant was present. The liver edge was palpable 3 cm. below the costal margin.

Examination of the blood revealed a hemoglobin of 13.9 gm. per cent and a white count of 17,400 per cu. mm. with moderate eosinophilia. A blood film showed mild hypochromia and toxic granulation. No spherocytes were present and the red cell fragilities were normal. The sedimentation rate was 67 mm. in the first hour. Abnormal pigments were absent from the plasma and the serum bilirubin values were normal. The fecal urobilinogen excretion was 10 mg. daily, consistent with the low values seen in severe diarrheal states. The patient's blood group was B, Rh positive (CDe/cde).

The bone marrow aspirate was hypercellular with 10 per cent eosinophilic myelocytes, and a myeloid: erythroid ratio of 6:1.

A 1+ direct Coombs test was noted. The serum contained a panantibody active against red cells treated with trypsin and ficin. The cold agglutinin titer was 1:4.

The gallbladder failed to visualize after a double dose of radiopaque material. By barium enema shortening of the colon with multiple areas of ulceration and small filling defects were demonstrated, the entire colon being involved except for the cecum and proximal ascending colon. Sigmoidoscopy revealed an edematous, profusely bleeding mucosa which on biopsy showed eosinophilic infiltration without arteritis.

The stools were grossly bloody, contained no ova or parasites. A trichinella skin test was negative. Agglutinin studies for enteric bacteria were also negative.

Shortly after admission symptoms in the right upper quadrant subsided. However, the patient continued to have as many as eight liquid, bloody bowel movements daily. In three weeks the hemoglobin fell to 9.0 gm. per cent, with a red cell count of 3.5 million per cu. mm.; hematocrit, 29 per cent; reticulocytes, 2.0 per cent; red cell indices, M.C.V. 82, M.C.H. 26, and M.C.H.C. 31; platelets, 274,000 per cu. mm.; and white cells, 9,400 per cu. mm. with 46 per cent neutrophils, 20 per cent band forms, 7 per cent eosinophils, 24 per cent lymphocytes and 3 per cent monocytes.

During the following months the patient received six transfusions, and for seven weeks was treated with antibiotics and symptomatic measures without improvement. ACTH gel was then administered for five weeks, followed by maintenance cortisone therapy. With hormonal therapy alone the hemoglobin level was maintained at 11 gm. per cent, with 2 per cent reticulocytes. Repeat sigmoidoscopy showed

decreased inflammatory changes in the mucosa. The patient was subjectively improved and was having fewer stools with only small amounts of gross blood when discharged in April, 1954. On hormonal therapy it was noted that although there was no change in the intensity of the direct Coombs reaction, the panantibody in the serum was no longer demonstrable.

Comment. As in Case I, this patient had intestinal bleeding at the time of discovery of the positive Coombs test. While hospitalized, the rectal bleeding became very marked and, consistent with this, the hemoglobin level fell. A 2.0 per cent reticulocytosis was present. The normal bilirubin level, and absence of marked reticulocytosis, methemalbumin, spherocytosis and erythroid hyperplasia of the bone marrow were points against a marked hemolytic process. The low fecal urobilinogen value was inconclusive in the presence of severe diarrhea. A red cell survival study could not be undertaken, due to persistent rectal bleeding. On hormonal therapy the intestinal bleeding decreased and, following transfusions, the hemoglobin was maintained at a constant level of 11.0 gm. per cent with low grade reticulocytosis. It is therefore impossible to state definitely whether maintenance of the hemoglobin level was due to an effect on a hemolytic process or directly on the colitis. However, the consensus was that probably no significant hemolysis was occurring in this patient whose direct Coombs reaction was only 1+ in intensity.

CASE III. Mrs. F. L. (No. 5075), a forty-four year old housewife, first became ill in 1937 when, at age twenty-seven, she noted abdominal cramps and frequent, bloody, bowel movements. Since then she has had several remissions and exacerbations, with hospitalizations in 1941 and 1946 during which the diagnosis of ulcerative colitis was corroborated by sigmoidoscopy and barium enema. Physical examination had been essentially negative. Treatment had been symptomatic. Two blood transfusions had been administered in 1941, and two in 1946, all without incident.

In December, 1948, while her colitis was quiescent, jaundice accompanied by severe anemia was noted. She received iron and two blood transfusions during the following seven months. The colitis remained inactive. However, in July, 1949, she was hospitalized because of thrombophlebitis, which subsided shortly after admission.

On physical examination the patient appeared acutely ill, pale and icteric. The liver and spleen were non-tender and enlarged to 2 cm. below their respective costal margins. The left calf was warm and tender, with edema of the ankle and a positive Homans' sign.

Blood examination revealed a hemoglobin of 6.0 gm. per cent; red cells, 1.65 million per cu. mm.; hematocrit, 18 per cent; reticulocytes, 72 per cent; red cell indices M.C.V. 113, M.C.H. 37, M.C.H.C. 33; platelets, 254,000 per cu. mm.; white cells, 12,000 per cu. mm. with 65 per cent neutrophils, 17 per cent band forms, 13 per cent lymphocytes, 2 per cent monocytes and 3 per cent myelocytes. One hundred thirty nucleated red cells per 100 white cells were present. The smear contained numerous spherocytes and polychromatophilic cells, marked basophilic stippling, and showed anisocytosis and poikilocytosis of the erythrocytes. Osmotic fragility was greatly increased. Hemolysis began at 0.72 per cent NaCl and was complete at 0.32 per cent NaCl.

The bone marrow aspirate was hypercellular, 75 per cent of all the cells being erythroid precursors.

The patient's blood group was B, Rh positive (CDe/CDe). The direct Coombs reaction was 4+. The serum contained a panantibody and anti-E, presumably due to isoimmunization by previous transfusions. The cold agglutinin titer was 1:8.

A red cell survival study using the modified Ashby technic revealed that 65 per cent of the transfused cells were destroyed in four days.

The indirect serum bilirubin was 2.9 mg. per cent. The direct van den Bergh reaction was positive, the prothrombin time was 18 seconds, control 12.5 seconds. Other laboratory findings including urinalysis and stool examination were normal.

At the time of hospital admission the patient's stools were formed and guaiac-negative. Aureomycin was administered for a short period, with recurrence of bloody diarrhea which persisted for almost the entire hospital stay. Over a three-week period the patient received 5,500 cc. of blood. Splenectomy was performed, followed by uneventful recovery. The spleen weighed 450 gm. and showed "active hyperemia as seen in hemolytic anemia."

One month postsplenectomy her hemoglobin was 10.1 gm. per cent, with 10 per cent reticulocytes, and the bilirubin level was normal. The fecal urobilinogen excretion was 246 mg. daily. She was discharged improved six weeks following surgery, still showing some occult blood in the stools.

She was well until March, 1953, when following an upper respiratory infection she was rehospitalized because of exacerbation of colitis. The hemoglobin was 12.4 gm. per cent. The direct Coombs test was 3+, the serum antibody content was unchanged. Sigmoidoscopy revealed ulceration and polypoid changes of the mucosa. Because of this and the recurrent activity of the disease, ileostomy and subtotal colectomy were performed.

Following discharge she improved, until July, 1953, when serum hepatitis developed, characterized by weakness, icterus and a tender liver enlarged to 6 cm. below the costal margin. The serum bilirubin level was 4.9 mg. per cent, chiefly direct reacting, and the cephalin flocculation test was three plus. She recovered with standard medical therapy. Hemoglobin was maintained at 12.5 gm. per cent throughout the entire period.

Her most recent admission to The Mount Sinai Hospital was in February, 1954, for a discharging

sinus tract opening on the abdominal wall. Physical examination also revealed a small incisional hernia, a rectal stricture and a well functioning ileostomy. The liver was not palpable.

The blood findings were: hemoglobin, 13.0 gm. per cent; red cells, 4.82 million per cu. mm.; hematocrit, 43 per cent; red cell indices, M.C.V. 89, M.C.H. 29, M.C.H.C. 32; white cells, 8,900 per cu. mm. with 43 per cent neutrophils, 8 per cent band forms, 41 per cent lymphocytes, 1 per cent eosinophils, and 7 per cent monocytes; platelets, 352,000 per cu. mm.; reticulocytes, 1.6 per cent. No spherocytes were present and the red cell fragilities were normal. No methemalbumin was present in the plasma. The urine urobilinogen excretion was less than 1 mg. daily. Neither urobilinogen nor occult blood was present in the ileostomy drainage. The bone marrow aspirate was normally cellular with a myeloid:erythroid ratio of 3:1. The direct Coombs reaction was only 1+. The panantibody and anti-E were still demonstrable in the serum. Blood chemical determinations were normal.

On March 1, 1954, an abdominoperineal resection was performed from which the patient made an excellent recovery. The pathologic report of the specimen was "acute and chronic ulcerative colitis with areas of atrophy and repair."

Summary and Comment. The seventeen-year course of this patient's ulcerative colitis has ranged the entire gamut of complications and management, with several remissions and exacerbations on medical therapy, finally requiring ileostomy and subtotal colectomy with subsequent abdominoperineal resection at age forty-three. During a two-year remission of colitis a severe autoimmune hemolytic process developed characterized by icterus, splenomegaly, severe anemia, extreme reticulocytosis, spherocytosis and a 4+ direct Coombs reaction, accompanied by pan- and iso-antibodies in the serum. Splenectomy was performed. At the present time, five years following splenectomy, most of the laboratory findings have reverted to normal except that the patient's red cells yield a one-plus direct Coombs reaction and the antibodies in the serum persist.

In contrast to the previous two cases there is no doubt in this instance about the genesis of the anemia which was present at the time the positive direct Coombs test was noted. The patient had been in clinical remission from her ulcerative colitis for more than two years and her stools were negative for occult blood when the 4+ direct Coombs reaction was noted. Therefore, blood loss could not be implicated as the cause of the severe anemia. The physical findings and laboratory data, including cell survival studies, indicated the presence of a severe autoimmune hemolytic process for which splenectomy was performed. Subsequently, due to reactivation of the colitis, ileostomy, subtotal colectomy and abdominoperineal resection were carried out. Following these surgical procedures the patient has been maintaining her hemoglobin at a high level and has normal laboratory findings except for the presence of antibodies in the serum and a 1+ direct Coombs reaction, despite which the hemolytic process has apparently subsided.

CASE IV. Mrs. L. B. (No. 26168) was first admitted in June 1951, at age seventeen, with a six-month history of mucous diarrhea, abdominal cramps and moderate fever. She had been treated symptomatically by her physician. Three months prior to admission migratory polyarthritis and erythema nodosum developed. At no time was gross blood noted in her stools. Despite this a normochromic anemia developed which was unresponsive to liver and iron therapy, and in May, 1951, the patient received one transfusion at another hospital. She continued to have about eight liquid bowel movements daily, and the following month was admitted to The Mount Sinai Hospital for the first time.

Physical examination revealed moderate wasting and pallor. There was no icterus. Other than a temperature of 101°F. the positive findings were confined to limitation of extension of both elbows, external hemorrhoids and the lesions of erythema nodosum on both legs.

Blood examination revealed a hemoglobin of 10.8 gm. per cent; red cells, 3.73 million per cu. mm.; hematocrit, 33 per cent; red cell indices, M.C.V. 88, M.C.H. 29, M.C.H.C. 33; white cells, 7,700 per cu. mm. with 29 per cent neutrophils, 49 per cent band forms, 18 per cent lymphocytes, 2 per cent eosinophils and 6 per cent monocytes; platelets, 300,000 per cu. mm.; reticulocytes, 0.8 per cent. The red cells were normochromic with slight anisocytosis and poikilocytosis, perhaps a reflection of the previous transfusion. No spherocytes were noted. The sedimentation rate was 82 mm. in the first hour. Urinalysis was normal. The stools were semiliquid, without gross blood, and gave either negative or 1+ reactions to guaiac reagent. Repeated examinations were negative for ova and parasites. Blood chemistry examinations including direct and indirect bilirubin were normal.

On admission, barium enema revealed diffuse changes of ulcerative colitis involving the entire colon, except for the rectum, which was also normal on proctoscopic examination.

Four weeks of therapy with intravenous ACTH resulted in disappearance of fever and erythema nodosum, a slight decrease in the frequency of bowel movements and accompanying well-being. Cessation of therapy resulted in reversion to the pretreatment state. Cortisone was then administered for six weeks, again with resultant improvement which was not maintained following cessation of therapy.

Surgical intervention was suggested in October, 1951, when a repeat barium enema failed to demonstrate improvement and proctoscopy revealed a friable, ulcerated anal mucosa. Ileostomy and subtotal colectomy were performed, prior to and during which 1,500 cc. of blood were administered. The

postoperative course was uneventful. The pathologic report was " . . . severe acute and chronic ulcerative colitis with a similar . . . milder process in the dissected segment of ileum." On discharge from the hospital the hemoglobin was 12.6 gm. per cent.

Thereafter the patient did well and gained thirty pounds in weight. However, in July, 1953, small amounts of blood and mucus were passed from the colostomy stump and rectum. This persisted for the following thirteen months and on August 2, 1954, she was readmitted for an abdominoperineal resection.

On physical examination the patient was in no distress and was not icteric. The ileostomy functioned properly. A small draining colostomy opening and two perineal fistulas were present.

Blood examination revealed a hemoglobin of 12.4 gm. per cent and a white count of 11,800 per cu. mm. with a normal differential. Urinalysis was normal and the ileostomy drainage contained no occult blood. Blood chemical determinations including direct and indirect bilirubin were normal. Proctoscopy revealed the anal mucosa to be very edematous and friable.

Due to difficulty in cross-matching blood for the projected surgery, detailed antibody studies were undertaken. The patient's blood group was B, Rh positive (CDe/cde), the direct Coombs test was weakly (±) but definitely positive. The serum contained an anti-E and a panantibody, both revealed only by ficin-treated red cells, and an anti-Kell, present to 1:256 titer. No cold agglutinin was present. The bone marrow was normally cellular with a myeloid:erythroid ratio of 3:1.

An abdominoperineal resection was performed, during and following which 1,500 cc. of blood were administered. The postoperative course was uneventful. Pathologic report of the specimen was chronic ulcerative colitis with areas of atrophy, scarring and hemorrhage. A postoperative blood count was: hemoglobin, 12.6 gm. per cent; red cells 4.6 million per cu. mm.; hematocrit, 41 per cent; reticulocytes, 1.0 per cent; red cell indices, M.C.V. 87, M.C.H. 26, M.C.H.C. 31; platelets, 236,000 per cu. mm.; white cells, 8,800 per cu. mm. with 66 per cent neutrophils, 7 per cent band forms, 24 per cent lymphocytes and 3 per cent monocytes. The red cells were slightly hypochromic. There were no spherocytes and the red cell fragilities were normal.

Comment. Despite the lack of gross blood in her stools during the first six months of colitis, anemia developed which was unresponsive to liver and iron and for which the patient received one transfusion. No icterus, splenomegaly, spherocytosis or reticulocytosis was present but no significant amount of occult blood occurred in her stools. Thus it is possible that the anemia may have been due in part to a hemolytic process as well as to chronic infection. Unfortunately, no direct Coombs test or antibody studies were performed at the time of ileostomy and subtotal colectomy.

Almost three years later, immediately prior to an abdominoperineal resection, the patient was noted to have a positive direct Coombs test and several antibodies in the serum. Despite these findings, however, no evidence for active hemolysis was found and the patient was not anemic. She had had no transfusions or other medications since the previous hospitalization and was not troubled by colitis except for minimal bleeding for one year prior to the discovery of the positive direct Coombs test. It is therefore possible that the positive direct Coombs test may have been present three years previously at the time severe anemia was noted.

DISCUSSION

The four cases presented have in common the association of severe ulcerative colitis and a positive direct Coombs test. The patients were all women, three white and one Negro, of various blood groups (O, cde/cde; B, CDe/cde; B, CDe/CDe; B, CDe/cde). In the first three patients the Coombs reactions were of varying intensities (1+, 3+, 4+), and did not appear to be related to the age of the patients (twenty-eight, forty-five, thirty-seven years), the previous duration of the colitis (one, one and one-half, eleven years), or the extent of colonic involvement. Immediately prior to the discovery of the positive direct Coombs reaction two of the patients (Cases I and II) had had episodes of active colitis of twelve months' and three weeks' duration, respectively. However, Case III was in remission when hospitalized for a severe autoimmune hemolytic process. Case IV should probably be considered separately as the Coombs test was first performed three years following ileostomy and subtotal colectomy, rather than earlier in the course of the disease when it may first have become positive.

The patients had received a comparatively small number of transfusions (four to ten), beginning from six months to eight years prior to the discovery of the positive antiglobulin test. Subsequently, additional transfusions were administered to all four and were well tolerated.

Jaundice and hepatomegaly accompanying the globulin-coated red cells was found only in Case III, although moderate hepatic enlargement was noted in Case II. No significant lymphadenopathy was evident in any of the patients.

In Cases I and II short courses of ACTH and cortisone given primarily for their possible effect on the colitis did not result in any change in the intensity of the Coombs reaction.

The concept of an immunologic mechanism as causative in some cases of acquired hemolytic anemia, first postulated and demonstrated by Chauffard and Vincent,[3] was re-emphasized by Dameshek and Schwartz.[4] With the discovery of the antiglobulin test[5] it became possible to detect incomplete Rh agglutinins in hemolytic disease of the newborn, supposedly due to adsorption of the globulin antibodies on the surface of the red cells. The application of the Coombs test to other hemolytic processes by Boorman et al.[6] permitted differentiation of congenital spherocytosis from acquired hemolytic anemia and demonstrated that the latter was an immune process.

Although coated erythrocytes can frequently be demonstrated in acquired hemolytic anemia, the question of whether globulin-coated red cells invariably have a shortened life span has been considered by several investigators. Mollison and Paterson[7] demonstrated by cell survival studies, using Rh positive cells previously incubated with serum containing Rh antibody, that most of the sensitized red cells survived normally when transfused into anemic patients. They postulated that a certain minimal quantity of antibody was needed for hemolysis. Loutit and Mollison[8] transfused coated red cells from a patient with acquired hemolytic anemia into a normal person and found that they had a normal life span. They postulated that a co-hemolysin in the plasma may be required in addition to the hemolysin on the red cell to result in a decreased red cell life span. Selwyn and Hackett[9] followed the survival of erythrocytes by the Ashby technic and performed "differential direct Coombs counts" to distinguish sensitized and unsensitized red cells. Blood from a patient with acquired hemolytic anemia was transfused into a normal patient and it was noted that there was rapid destruction for ten to fifteen days, followed by a normal rate of destruction with a normal total life span. They too, concluded that sensitization *per se* does not necessarily mean shortened survival, and postulated that in the initial rapid destruction perhaps only the most heavily coated cells were destroyed; those less heavily sensitized surviving normally. Abnormal hemolysis may thus be a threshold phenomenon.

The activity of the hemolytic process also was thought to be related to the amount of antibody on the red cell by Evans and Duane.[10] Others,[11] however, report little if any correlation between autosensitization and autohemolysis. In the four cases with sensitized red cells herein reported hemolysis did appear to be related to the degree of sensitization. The only case with a frank hemolytic process was Case III whose direct Coombs reaction was 4+. The anemia present in the other three cases probably had no significant hemolytic component. In splenectomized patients, such as Case III, coated erythrocytes often persist even though all evidence of accelerated hemolysis has subsided.[10,12,13] In such patients[13] the spleen probably is a major hemolytic organ, although only a minor source of antibody.

Evidence has been presented that antibody formation in response to specific antigenic stimuli may occur in the reticuloendothelial system,[14] spleen,[15,16] lymph nodes[17–19] or plasma cells.[20,21]

In "idiopathic" acquired hemolytic anemia the stimulus is unknown. Viruses,[22] haptene complexes composed of a component of the red cell plus a foreign substance such as a virus or drug,[18] free fatty acids or enzymes[23] or previous transfusions, have all been implicated. It has been suggested that the action of the agent may be to modify the erythrocyte surface structure, thereby rendering it antigenic.[15] Experimentally antibody-coated red cells have been produced following repeated intraperitoneal[23] or subcutaneous[24] injection of homologous blood, which in some instances had been modified by Freund's adjuvant or by exposure of the red cells to influenza virus.

In the symptomatic hemolytic anemias the underlying condition is presumably responsible for the hemolytic process.[25–27] The mechanism may be that of direct injury of the erythrocytes or alteration of their surface structure[27] stimulating the production of autoantibodies of low specificity which affect normal red cells as well as the altered ones. In the hemolytic process often associated with the lymphomas[7,10,11,29] the tissue itself may produce abnormal autoantibodies which coat the patient's red cells.[11] In the "reticuloses" it has been postulated that "the abnormal tissues may destroy excessive numbers of red cells, and thus provoke hemolytic antibody production."[28]

In ulcerative colitis there are alterations in the absorption of fat, carbohydrates and amino acids by the small intestine.[30] It is possible that in this disease certain normally rejected substances are absorbed by the colon or by the

small intestine. These substances may possess antigenic properties in common with the erythrocytes, thus stimulating the formation of red cell antibodies. Similarly, the diseased colon itself may have a haptene in common with the erythrocytes which might stimulate the production of antibodies capable of coating the red cells.

While the mechanism of antibody elaboration is unknown, it nevertheless appears that the occurrence of positive direct Coombs reactions in ulcerative colitis is more than fortuitous and is probably related to the presence of diseased bowel although independent of the activity of the colitis. Case III had been in clinical remission from colitis for more than two years at the time the severe autoimmune hemolytic process became manifest. Although splenectomy had induced a remission in the hemolytic process, the 4+ intensity of the Coombs reaction decreased only slightly in a four-year period. Within one year after ileostomy and subtotal colectomy, however, it had decreased to 1+. Thus even though gross hemolysis had ceased with removal of the spleen, there was not much change in the amount of antibody coating the red cells until the diseased colon had been removed. In Case IV, unfortunately, no direct Coombs test was performed at the time the patient was found to have been severely anemic, despite the lack of significant amounts of occult blood in her stool and the short duration of her illness. A slightly positive direct Coombs reaction was recently noted, almost three years later. At this time the patient was no longer anemic and had no evidence of hemolysis. By analogy with Case III it is very possible that at the earlier hospital admission the patient had a strongly positive direct Coombs test which over the intervening years, subsequent to ileostomy and subtotal colectomy, diminished to its present intensity. In Case I only seven months have elapsed since ileostomy and subtotal colectomy. However, the patient now has no anemia or reticulocytosis and there has been a slight but definite diminution in the intensity of the direct Coombs reaction. If the diseased bowel plays a role in initiating or maintaining the antibody coating of the erythrocytes, with the passage of time its removal should result in a further diminution of the intensity of the direct Coombs reaction, as happened in Case III and as may have occurred in Case IV. Whether the ileostomy, with its diversion of fecal material which may contain antigenic substance that might otherwise be absorbed, or the subtotal colectomy, resulting in the removal of diseased bowel which may have haptenes in common with the erythrocytes, is the more important in causing diminution of the direct Coombs reaction cannot as yet be stated.

SUMMARY

Four cases of ulcerative colitis associated with antibody-coated red cells are presented. The clinical, hematologic and immunologic findings are discussed.

One of the patients had overt hemolytic anemia of the acquired autoimmune variety. It was not possible to assess the role played by hemolysis in the anemia presented by the other three patients.

The occurrence of antibody-coated red cells in ulcerative colitis appears to be related to the underlying intestinal disorder, and particularly to the presence of diseased bowel, although it is not necessarily related to the clinical activity of the colitis.

The possibility of an autoimmune hemolytic process should be considered in the pathogenesis of anemia in ulcerative colitis.

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Prostatic Cancer*

XII. Extremely Elevated Serum Acid Phosphatase Associated with Altered Liver Function

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VALUES above a statistically established upper limit for the normal acid phosphatase activity in serum are almost always indicative of metastatic adenocarcinoma of the prostate gland.[1] In such instances the source of additional enzyme present in the serum is believed to be the secretion of the prostatic acini which form the metastases; such glandular structures, having no prostatic ductile outlet comparable to the normally situated gland, pour acid phosphatase into the surrounding tissue. The enzyme, being freely diffusible, then appears in increased amounts in the blood.

Such a convenient and apparently acceptable explanation of the sequence of events which lead to an increase in the quantity of enzyme in the blood leads one to speculate about the mechanism by which acid phosphatase is eliminated or destroyed. The excretion of alkaline phosphatase in the bile seems an established fact[2] but knowledge of the fate of prostatic acid phosphatase is lacking. It has been assumed that the acid phosphatase of the urine in man is largely derived from prostatic washings by the urine and merely represents the enzyme added by the normal "resting" prostatic secretion.[3] Urinary acid phosphatase of prostatic origin is not increased by the development of metastases from prostatic cancer, even in the presence of an elevated serum level.

The present report is concerned with the coexistence of enormously increased amounts of serum acid phosphatase and extensive metastases of the liver from prostatic cancer, and with a possible cause and effect relationship. A study of this relationship in three patients will be described.

CASE REPORTS

CASE 1. C. B. (Presbyterian Hospital Unit No. 825,045), a sixty-four year old white man, was admitted to the hospital on April 12, 1946, for relief of acute urinary retention which had developed during a one year period of symptoms suggesting prostatic obstruction. No history of cardiorenal, hepatic or other systemic disease was present. A diagnosis of prostatic cancer was made by rectal palpation. Initial treatment consisted of enucleation prostatectomy, bilateral orchiectomy and commencement of daily oral dosage of 5 mg. of diethylstilbestrol. The surgical-pathologic specimen provided a confirmatory diagnosis of adenocarcinoma of the prostate gland, well differentiated. During this hospitalization neither radiographic nor serum acid phosphatase studies were indicative of metastatic disease. The patient, symptomatically improved, was discharged on June 9, 1946.

On September 24, 1951, this man was again admitted to the hospital in acute urinary retention. The rectal palpation appraisal at this time was suggestive of local extension of a neoplasm originating in the prostate gland. The serum acid phosphatase level on October 3, 1951 was 4.2 Gutman units. There was no evidence by x-ray of osteoblastic metastases. The patient was relieved of 800 cc. of residual urine and treated by continuous gravity drainage by means of an indwelling Foley bag urethral catheter. The diethylstilbestrol oral dosage was increased to 500 mg. daily. The urethral catheter was removed prior to the patient's discharge from the hospital on October 10, 1951, at which time urethral voiding was abnormal only in that

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it was frequent and characterized by a weak urinary stream.

On January 3, 1952, the patient was readmitted with a primary complaint of sudden appearance and progressive enlargement of an epigastric mass, with concomitant asthenia, anorexia and constipation. For the first time he complained of intermittent, low back pain. The lower edge of the non-tender liver was palpable 5 cm. below the right costal margin. The spleen was also found to be enlarged on palpation. There was bilateral ankle edema. Little change in observations at rectal palpation of the prostate gland was noted. The serum alkaline phosphatase level was elevated to 15.4 units (Bodansky) and the acid phosphatase to 201 units (Gutman). The cephalin flocculation test was 2+ and the thymol turbidity test 3+. The albumin:globulin ratio was 2.6:2.4. The blood urea nitrogen, sodium and potassium were within normal limits. Fourteen days after this readmission jaundice became evident, persistent dark urine and light stools developed, ascites accumulated and dependent edema increased. The alkaline phosphatase rose to 18.4 units, and the acid phosphatase to 319 units. A small area of increased density appeared on x-rays in the left side of the bony pelvis. By the twenty-fifth hospital day the patient was semicomatose, and on January 27, 1952 he died.

The significant observations noted at autopsy were confined to the lungs, liver, urinary bladder, prostate gland, the lymphatic system and the red bone marrow. In the lungs tumor cells in the lymphatics, blood vessels and lung parenchyma were present. This process was extensive. In the liver the hepatic architecture was completely destroyed. Extensive areas of necrosis and infiltration by inflammatory and tumor cells were noted. The urinary bladder was dilated and trabeculated; although inflammatory changes were present in the subepithelial layer, no invasion by neoplastic cells occurred. Large areas of necrosis in the prostatic bed with extension into the surrounding perineal and pelvic structures were present. There were some areas of well differentiated neoplastic cells. Tumor infiltration extended into the bladder neck. Lymph glands in the cervical, thoracic and abdominal regions were almost completely replaced by tumor tissue. The glandular capsules were the only remnants of the normal architectural structure. Of special interest was the bone marrow: throughout the examination of the red marrow system only one microscopic section of all those taken showed any tumor tissue whatever.

Case II. J. S. (Francis Delafield Hospital No. 2878), a seventy-seven year old white man was admitted to the Francis Delafield Hospital on May 5, 1953, for terminal care; a diagnosis of metastatic carcinoma of the prostate had been made. At another hospital a serum acid phosphatase of 16.3 Bodansky units, alkaline phosphatase 25.1 Bodansky units, albumin 3.8 gm. per cent and globulin 2.6 gm. per cent were recorded. Upon admission to the Delafield Hospital the patient was confused and disoriented. Physical signs of a left pleural effusion, confirmed by x-ray, were noted. The liver edge was palpable five fingerbreadths below the right costal margin in the mid-clavicular line; it was hard but no nodules were palpated. Jaundice was not present. The prostate was stony hard and fixed. Blood studies of May 7, 1953 revealed an acid phosphatase of 475 Bodansky units, alkaline phosphatase 24.7, serum bilirubin 0.2 mg. per cent, thymol turbidity 2.1 units, cephalin flocculation test 1+ and hemoglobin 6.5 gm. No bile was present in the urine. X-ray studies at this time showed osteoblastic and osteolytic metastases in the lumbar spine, ribs and shoulder girdle. A chest x-ray film demonstrated, in addition to a left pleural effusion, diffuse peribronchial infiltration.

Within the following week the patient was given transfusions of two liters of whole blood. Several days thereafter he became increasingly dyspneic and disoriented. Liver function tests were repeated on May 29. The thymol turbidity test was 4.5 units, cephalin flocculation test 2+, serum bilirubin 0.4 mg. per cent, bromsulfalein 14 per cent retention in forty-five minutes and prothrombin time 19.4 seconds (normal 11.4). The serum acid phosphatase was 400 Bodansky units and the alkaline phosphatase 32.3 Bodansky units. By June 2 jaundice was observed clinically and the patient became cyanotic. Digitalis was administered, mercurial diuretic drugs were given and the patient was placed in an oxygen tent. On June 3, after several convulsive seizures, he died. No autopsy was performed.

Case III. C. W. (Francis Delafield Hospital No. 3202), a fifty-two year old Negro man, a laborer, was admitted to the Francis Delafield Hospital on November 6, 1953. For five months he had complained of sacral and bilateral lower extremity pain and for three to four weeks of

generalized pruritus. At the time of the patient's admission to the hospital moderate jaundice developed but the liver was not palpable. Examination of the rectum revealed a stony hard, fixed prostate. A radiographic skeletal survey revealed widespread osteoblastic lesions. A serum acid phosphatase of 550 Bodansky units and an open perineal biopsy confirmed a diagnosis of metatastic prostatic carcinoma.

Laboratory studies during hospitalization gave the following positive results: serum bilirubin 7.5 mg. per cent, prothrombin time 18.9 seconds (normal 14.8), cephalin flocculation test 2+ and thymol turbidity test 3+. Albumin: globulin 3.3:3.4 gm. per cent and alkaline phosphatase 23.4 Bodansky units.

On December 24, 1953, under continuous caudal anesthesia, bilateral orchiectomy was performed. Immediately postoperatively the patient was given 500 mg. of diethylstilbestrol a day. Within one month he was free of pain and had gained 10 pounds. During this period the jaundice disappeared, prothrombin time returned to within normal limits, serum bilirubin was 0.4 mg. per cent, cephalin flocculation test 1+, the thymol turbidity test gave negative results, albumin:globulin was 4.1:3.4 gm. per cent, alkaline phosphatase 25.4 and acid phosphatase 1.0 Bodansky units. The patient was discharged February 1, 1954, and has continued to do well.

EXPERIMENTAL STUDIES

In spite of extensive literature concerning the elevation of acid phosphatase in the sera of patients with metastatic carcinoma of the prostate, precise identification of the enzyme (or enzymes) responsible for this elevation does not appear to have been established, although Gutman and Gutman provided evidence that it is of prostatic origin.[4] For this reason a study of the qualitative nature and the quantitative level of the acid phosphatase of various body fluids and tissues (where feasible) was made. Results of such investigations will be presented in the following sections. Source material for all the experiments listed, unless otherwise specified, was derived from the patient discussed in Case 1 (C. B.). The material was considered especially well suited for these investigations in view of the exceptionally high concentration of acid phosphatase present within the serum of this patient. Results of kinetic studies done on the sera of the other patients listed in this report were qualitatively similar to those results derived from the study of material of patient C. B. and have therefore not been included as a part of this report. The increased serum acid phosphatase observed in these patients was concluded to be, in fact, of prostatic origin on the basis of kinetic evidence which has been summarized in detail in the following sections.

Preparation of Source Materials for Enzyme Studies. The serum, in addition to being tested directly after appropriate dilution, was subjected to ammonium sulfate fractionation for further investigation. The bulk of the enzyme, representing approximately 85 per cent of the original total, was recovered between 0.63 to 0.67 saturation with ammonium sulfate (at 23°c.) in the presence of 0.1 M acetate buffer at pH 5.5. The resulting enzyme fraction was estimated to represent an approximate eightfold purification on the basis of tyrosine measurements using the reagent of Folin and Ciocalteau.[5]

Approximately 5 gm. of the primary neoplastic tissue were removed and homogenized with 9 volumes of water. It was necessary that the tissue, because of its fibrous nature, be cut into small pieces prior to homogenization. The resulting homogenate was centrifuged for ten minutes at 23,000 × g. The sediment was found to contain no enzyme and was discarded. The remaining supernate was used for the enzyme measurements.

Approximately 5 gm. of hepatic metastatic tissue were selected and removed. The tissue was homogenized with 9 volumes of water and used directly for the enzyme measurements.

Approximately 2.5 gm. of the osseous metastases were selected and removed. The tissue was thoroughly pulverized and ground with a mortar and pestle, after which 9 volumes of water were added. The resulting suspension contained no gross bone fragments. It was lightly centrifuged (2,500 × g for ten minutes) and the resulting supernate was taken for the enzyme measurements.

The urine sample (235 ml.) was tested directly for enzyme.

Enzyme Measurements. Reliable methods for the accurate measure of prostatic acid phosphatase using various substrates were previously developed in connection with other investigations and have been applied in these studies. Only a brief description of the methods will be presented. The methods as applied have incorporated optimum conditions for enzymatic

activity with respect to substrate concentrations (that is, saturating levels in each instance), hydrogen ion concentration and incubation time. All assays were done on at least two levels of enzyme concentration to insure strict proportionality between enzyme concentration and activity.

Commercial preparations of phenylphosphate, beta-glycerophosphate and yeast adenylate of high purity served as enzyme substrates in the differential study. Details concerning purification procedures and relative purity of each substrate have been presented elsewhere in connection with other studies.[6] Each of the substrates was prepared at high concentrations and adjusted to appropriate hydrogen ion concentration prior to use (due to inherent buffering properties).

Stock solutions of molar acetate buffer were prepared to cover the pH range from 4.0 to 6.0 and were used at a final 0.1 M concentration within the reaction mixtures.

All enzyme measurements were carried out in a final 1 ml. reaction volume. Larger volumes were avoided in order to maintain high concentrations of substrate (that is, saturating levels) while limiting the absolute quantities within the reaction mixtures. The presence in excess of a specific amount of two of the three substrates (beta-glycerophosphate and yeast adenylate) was found to interfere markedly with the colorimetric determination of inorganic phosphorus.[6] Tubes were prepared in advance containing substrate, buffer and water (if required) to 0.5 ml. volume. To the prepared tubes 0.5 ml. volume of appropriately diluted enzyme solution was added. The reaction tubes were incubated at 37°c. for a thirty and sixty minute period to insure strict proportionality between incubation time and enzyme activity with each new sample investigated. Following the incubation period, the reaction was stopped by the addition of 5 ml. 10 per cent trichloroacetic acid. The contents of the tubes were filtered (if required) through acid-washed paper and aliquots of the filtrate removed for inorganic phosphorus determination.[7]

RESULTS

Serum Studies. The marked elevation of serum acid phosphatase found on routine laboratory study was readily confirmed. Table 1 summarizes the measurements of the acid phosphatase activity on the serum. The enzyme was tested at three dilutions in the presence of saturating levels of three separate substrates. The reliability of the assay methods is demonstrated by the strict proportionality obtained between enzyme concentration and activity in each of the substrates used. All enzyme assays were made at pH 5.5 in 0.1 M acetate buffer after preliminary experiments had indicated such an acidity to be most nearly the average optimum for the three substrates (each substrate showing a slightly different pH optimum). A measure of the serum for acid phosphatase activity under optimal (or near optimal) conditions showed hydrolysis of 1250 mg. P per hour per 100 ml. serum using saturating levels of phenylphosphate. The hydrolysis of 1250 mg. P corresponds to the simultaneous release of 3790 mg. phenol. From the clinical report, 319 mg. phenol were calculated to have been hydrolyzed per hour per 100 ml. serum by using the assay procedure of Gutman and Gutman,[8] approximately twelve times less than observed by our methods; the discrepancy between the two levels is undoubtedly the result of improper dilution in the initial assay with a consequent error in this unusually high range of values.

TABLE 1

QUANTITATIVE MEASURE OF ACID PHOSPHATASE ACTIVITY ON THREE DIFFERENT SUBSTRATES WITHIN THE SERUM SAMPLE UNDER INVESTIGATION

Dilution of Serum*	Substrate†		Phosphorus Liberated/hr. at pH 5.5‡		
	Compound	Concentration (molar)	Per Reaction Vessel (μg)	Per ml. Serum (calculated) (mg.)	Per 100 ml. Serum (calculated) (mg.)
1:500	Phenylphosphate	0.138	12.4	12.4	1240
1:250			25.3	12.7	1270
1:125			49.6	12.4	1240
1:500	β-glycerophosphate	0.128	13.6	13.6	1360
1:250			27.2	13.6	1360
1:125			52.0	13.0	1300
1:500	Yeast adenylate	0.066	10.1	10.1	1010
1:250			20.9	10.5	1050
1:125			41.2	10.3	1030

* Dilutions made in distilled water; to the reaction mixtures was added 0.5 ml. of the diluted serum for enzyme measurements.

† Substrates were prepared as concentrated stock solutions adjusted to pH 5.5. To the reaction mixtures was added 0.2 ml. of 0.69 molar phenylphosphate, 0.2 ml. of 0.64 molar beta-glycerophosphate and 0.3 ml. of 0.22 molar yeast adenylate, respectively. The concentrations of substrate used represent saturating levels in each instance.

‡ Phosphorus released by enzyme cleavage (that is, in excess of that contributed as contaminants by the substrates and serum) was determined after one hour incubation at 37°c. in the presence of 0.1 molar acetate at pH 5 5. All final reaction volumes were 1 ml.

The serum acid phosphatase was found to hydrolyze beta-glycerophosphate at a rate comparable to that observed with phenylphosphate. The action of the enzyme on yeast adenylate was somewhat less than that observed with the other two substrates tested. The pattern of action

TABLE II
COMPARATIVE ACTION OF PROSTATIC ACID PHOSPHATASE IN PRESENCE OF SATURATING LEVELS OF PHENYLPHOSPHATE, BETA-GLYCEROPHOSPHATE AND YEAST ADENYLATE

Enzyme Preparation*	Substrate†		Acid Phosphatase Activity‡
	Compound	Concentration	
Crude prostate homogenate	Phenylphosphate	0.138 M	43.0
	β-glycerophosphate	0.128	45.2
	Yeast adenylate	0.066	37.4
Partially purified prostatic acid phosphatase	Phenylphosphate	0.138 M	404
	β-glycerophosphate	0.128	412
	Yeast adenylate	0.066	370

* Crude homogenate prepared from benign hypertrophic prostate gland by grinding finely sliced pieces in a glass homogenizer. Partial purification of the enzyme (approximately tenfold on the basis of nitrogen) accomplished by ammonium sulfate fractionation and dialysis of crude homogenate.

† Substrate concentrations represent saturating levels in each instance.

‡ Acid phosphatase activity refers to mg. P hydrolyzed/hr./mg. N at pH 5.5 and 37°c.

of the serum enzyme on each of the substrates was found to be identical with that observed with an acid phosphatase obtained from prostatic tissue. For purposes of comparison a typical measure of prostatic acid phosphatase using crude and partially purified enzyme preparations is presented in Table II. A tentative identification of the serum acid phosphatase as being in fact of prostatic origin was made on the basis of this evidence.

Further Studies on Serum Acid Phosphatase Following Partial Purification. A partial purification of the serum acid phosphatase was accomplished by ammonium sulfate fractionation, approximately eightfold on the basis of nitrogen content. The resulting enzyme preparation was subjected to intensive characterization with respect to its kinetic properties. The details concerning these investigations will be presented elsewhere[9] and only a brief summary of the results will be presented here.

The serum enzyme was found to be identical with the prostatic acid phophatase in its relationship between reaction velocity and substrate concentration, hydrogen ion and specific inhibitors (including fluoride and tartrate). Perhaps the most convincing evidence for the identity of the two enzymes is furnished by the observed action on the three separate substrates (phenylphosphate, beta-glycerophosphate and yeast adenylate). From the substrate studies the dissociation constants (K_s) for each of the substrate-enzyme complexes were determined. The K_s values for the serum enzyme were as follows: for phenylphosphate, 3 by 10^{-4} M; for beta-glycerophosphate, 4 by 10^{-3} M; and for yeast adenylate, 5 by 10^{-4} M. These values do not differ greatly from those with highly purified preparations of acid phosphatase (approximately three hundred fold based on nitrogen) from prostatic tissue.[10] Positive identification of the serum enzyme as being of prostatic origin would appear to be convincingly established on the basis of this experimental evidence.

Acid Phosphatase Composition of Primary Prostatic Neoplasm, Hepatic and Osseous Metastases and Urine. Additional investigations were carried out on postmortem material to determine the quantitative as well as qualitative distribution of acid phosphatase within different tissues. In Table III are summarized the results of the acid phosphatase measurements. The pattern of action of the enzyme associated with each of the samples of material tested strongly implicates the acid phosphatase to be of prostatic origin in each instance. Among the tissues investigated, the hepatic metastasis was found to contain the greatest concentration of enzyme. The primary prostatic neoplasm contained the least. The primary tumor was found to contain less than 3 per cent of the acid phosphatase present in a sample of non-neoplastic prostate. (Compare with results summarized in Table II.) No significance can be attached to the relative values of prostatic acid phosphatase for the metastatic tissue analyzed.

Additional experiments were performed on normal human liver for purposes of comparison with hepatic metastasis. Normal human liver was found to contain an acid phosphatase of distinctly dissimilar kinetic properties and in concentrations (assuming activity to be representative of concentration) of less than 4 per cent of the enzyme found to be present within the metastatic tissue. Studies concerning the specific kinetic properties of human liver acid phosphatase will be presented elsewhere in relation to other investigations which are prompted by liver acid phosphatase studies

reported by Davies[11] and by Bamann and Riedel.[12]

COMMENTS

The differential substrate experiments here described were considered to establish beyond any reasonable doubt that the enzyme found in the body fluids and tissues was of prostatic glandular origin. Hepatic metastases of prostatic origin of recognizable degree are uncommon. Extensive involvement of the liver by prostatic cancer such as described here is rare.

The total volume of metastatic glandular tissue in Case I is less than that in many other instances of prostatic cancer in which extremely high serum acid phosphatase levels are not detected at any time during the course of the disease. The clinical and laboratory evidence of progressive hepatic insufficiency in Case I directly paralleled the sharply rising serum acid phosphatase. In Case II the rapid fall of serum acid phosphatase following bilateral orchiectomy was accompanied by a return to normal reactions of the liver function tests. Although it cannot be stated unequivocally that patient J. S. (Case II) had extensive metastatic involvement of the liver, the presumptive evidence for hepatic spread is strong.

There are at least two possible explanations for the high level of acid phosphatase in the sera of these patients. It is conceivable that prostatic carcinoma cells in an extremely vascular organ such as the liver can more easily secrete acid phosphatase into the systemic circulation. On the other hand those unknown metabolic processes concerned with the destruction of acid phosphatase may be impaired. It is probable that the liver is in some way responsible for the catabolism of serum acid phosphatase and that injury to this organ may impair this function.

TABLE III

DISTRIBUTION OF ACID PHOSPHATASE WITHIN PRIMARY NEOPLASM, HEPATIC AND OSSEOUS METASTASIS AND URINE SPECIMEN

Additional Material Investigated	Substrate*		Mg. N per† gm. Wet Weight Material	Mg. P Hydrolyzed/hr. at pH 5.5‡		
	Nature	Conc.		Per ml. Sample	Per gm. Wet Weight Material	Per mg. Nitrogen
Primary prostatic neoplasm....	Phenylphosphate	0.138M	8.3	....	81.6	0.98
	β-glycerophosphate	0.128	....	....	82.0	0.99
	Yeast adenylate	0.066	....	....	68.1	0.82
Hepatic metastasis.....	Phenylphosphate	0.138 M	20.7	....	333	16.1
	β-glycerophosphate	0.128	....	....	331	15.9
	Yeast adenylate	0.066	....	....	260	12.6
Osseous metastasis.....	Phenylphosphate	0.138 M	9.6	....	18.0	1.88
	β-glycerophosphate	0.128	....	....	16.4	1.71
	Yeast adenylate	0.066	....	....	15.1	1.57
Urine specimen.....	Phenylphosphate	0.138 M	....	4.64	....	....
	β-glycerophosphate	0.128	....	4.40	....	....
	Yeast adenylate	0.066	....	4.16	....	....

* Substrate concentrations represented saturating levels in each instance.

† Nitrogen was determined by micro-Kjeldahl.

‡ The reaction mixtures contained, in addition to tissue and substrate, 0.1 ml. molar acetate buffer at pH 5.5; all in a final 1 ml. reaction volume.

SUMMARY

1. The clinical and laboratory course of three patients with metastatic prostatic carcinoma and impaired hepatic function is described. In each instance there was an extremely elevated level of serum acid phosphatase activity.

2. The acid phosphatase measured in these studies was shown to be of prostatic origin.

3. In Case I the rise in serum acid phosphatase paralleled the increase in hepatic damage.

4. Case III demonstrated a parallelism between the fall of acid phosphatase after hormonal therapy and a return of liver function to normal.

5. The liver appears to be implicated in the metabolism of serum acid phosphatase of prostatic origin.

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LAKESIDE GENERAL HOSPITAL

Review

Melanin Pigmentation*

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WITH increased knowledge of the cytology of melanocytes, and of the enzymic, hormonal and neurogenic factors controlling their activity, it has become possible to formulate a unified concept of melanin pigmentation in man. In the presentation of this concept, basic cytologic and biochemical factors will first be considered and this will be followed by a description of common variations in pigmentation, including changes from birth to old age.

1. Cytology of Melanocytes. Melanin is formed in the cytoplasm of melanocytes by oxidation of the amino acid tyrosine in the presence of the enzyme tyrosinase. The term clear cell refers to the perikaryon of the melanocyte. The immature pigment cell is known as a melanoblast and the non-specific macrophage which phagocytizes melanin as a melanophage. Benign proliferation of melanocytes results in formation of a nevus. Malignant proliferation of melanocytes produces a melanoma.

In general, melanocytes are believed to arise embryologically from the neural crest,[1,2] although there are still a few adherents to the theory of epithelial origin.[3] Melanocytes are present in the skin, eyes and leptomeninges. Several technics can be used to visualize these cells. They may be stained with silver, gold or methylene blue, or visualized histochemically following incubation in solutions of tyrosine or dopa.[4,5] Tyrosinase, present in the cytoplasm of most melanocytes, catalyzes the oxidation of tyrosine or dopa to the dark pigment melanin. This process is called the tyrosine reaction or dopa reaction depending upon whether tyrosine or dopa is used as the substrate.

Although the melanocytes of skin, eyes and leptomeninges probably are derived from the neural crest, these cells differ from one another in several respects. Furthermore, within one structure, such as the skin, the melanocytes at the epidermal-dermal junction differ from those in the hair bulb or dermis. Usually, although there are many exceptions, the color of skin, hair, eyes and leptomeninges is related; a person with dark skin has dark hair, eyes and leptomeninges, whereas the fair-skinned person has light hair, eyes and leptomeninges.

In skin, melanocytes are present in the basal layer of the epidermis at its junction with the dermis and in the hair bulbs. These cells have numerous dendritic processes. Melanocytes may occur in the dermis as dermal nevi. Basal layer melanocytes are not distributed uniformly over the body More are found in the upper portions, on the face and forehead and behind the ears, than in the lower regions such as the thighs.[4,6] The palms also have relatively few pigment cells. Different races, such as Caucasian and Negro, have the same number of melanocytes in any particular location although their activity varies. With the exception of melanocytes from albinos or from depigmented areas of patients with vitiligo, skin melanocytes give a positive dopa reaction. The latter is increased when skin is treated by any method which increases the formation of melanin, for example, ultraviolet light radiation. Melanocytes from normal skin give a negative or weak tyrosine reaction.[6,7] However, exposure of skin to ultraviolet light modifies the melanocytes so that a positive tyrosine reaction can be obtained. Most nevi arise from basal layer melanocytes. Melanomas arise from melanocytes in apparently normal skin or from those in nevi. These tumors are highly malignant and metastasize widely.

Melanocytes of the hair bulb differ from those of adjacent skin in being more or less active. For example, dark hair often grows out of very light skin. In this case the melanocytes of the hair bulb are active and those in the skin are

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relatively inactive. When a patient with vitiligo who is being treated with psoralen compounds is exposed to ultraviolet light, repigmentation is seen first about the hair follicles.[8] Gradually, the pigmented portions of adjacent follicular openings enlarge and coalesce. On the other hand, gray hairs may protrude from dark skin. Here the skin melanocytes are more active than those from the hair. It is possible that the differences between melanocytes of the skin and hair bulb cells are due to the influence of surrounding structures. Grafting experiments show that removal of the epidermis and upper dermis but allowing the hair bulb cells to remain intact results (after healing) in normally pigmented skin.[4] Hence, basal layer melanocytes arise from hair bulb cells. On the other hand, when superficial epidermis is removed from an area of white hair in a guinea pig and replaced by basal layer epidermal cells from an area of pigmented skin, many new hairs grow out black instead of white.[4] In this case the hair becomes pigmented by the new melanocytes.

So far as is known, melanomas are not derived from hair bulb cells. However, this is a difficult point to study because a melanoma that did arise from a hair bulb might in its early stages of development look like a pigmented nevus on the skin surface.

In the eye, melanocytes normally occur along the uveal tract and in the retina. The cells of the uveal tract are similar to those of the skin in that thay have many dendritic processes. However, it is thought that melanocytes of chick and rabbit eyes differ from those of skin by giving a positive dopa reaction before birth but a negative reaction after birth.[9] These findings are not in accord with the fact that the human eye darkens with age, thus indicating the formation of new pigment. Beef ciliary bodies probably give positive dopa and tyrosine reactions because extracts from this tissue readily catalyze oxidation of tyrosine and dopa to melanin.[9] In fish and possibly in human beings sectioning sympathetic fibers results in hyperpigmentation of the denervated tissue because of dispersion of melanin granules within the cell.[10,11] However, cervical sympathectomy in man, followed by development of the Horner syndrome, causes no change or decrease in eye color. This phenomenon is contrary to that observed in skin. A further difference between eye and skin melanocytes is that melanomas which arise from the eye metastasize at a much slower rate than those from skin. The prognosis is better for the eye melanoma.

The retina contains dark pigment granules which have been referred to as melanin and which occur in cells arranged in palisade formation. It is not known whether these cells represent melanocytes. There have been no reports of melanomas arising from these retinal cells. Pigmentation of frogs kept in the dark is increased in the skin but decreased in the retina. When these animals are allowed to remain in the light their skin lightens while the retina darkens. Thus the dispersion of melanin granules in skin melanocytes proceeds oppositely from that in the retinal pigment cells.

The melanocytes of the leptomeninges tend to be bipolar.[12] At times large nevi on the face have been associated with excessive numbers of melanocytes in the leptomeninges on the involved side.[13] Melanomas arise less commonly from these melanocytes than from those of skin or eyes. The metastatic tendency is difficult to evaluate because the patient usually dies from destruction of vital centers due to local enlargement of the tumor.

It would be interesting to transplant melanocytes from eyes or leptomeninges into skin in order to observe their development and learn whether they assume the form of skin melanocytes.

Once melanin is formed in the melanocyte, it is eliminated usually through the skin surface by being passed through the epidermis and shed with the stratum corneum. Melanin also may be absorbed by the lymphatics. In normal skin, in which melanin is constantly being formed and eliminated, depigmentation may be produced by local application of monobenzylether of hydroquinone for a minimum time of three weeks. This suggests that once melanin formation is inhibited, three weeks are required for elimination of the melanin already present in the skin. However, in some lesions, such as café au lait spots or postsunburn freckles, melanin granules may be permanently dispersed and firmly fixed within the cell.

2. Enzymic Factors in Melanin Formation. Melanin is formed by oxidation of the amino acid tyrosine in the presence of the enzyme tyrosinase. Tyrosinase is a copper enzyme complex located in mitochondrial elements in the cytoplasm of melanocytes. Mammalian tyrosinase is a more specific enzyme than that derived from plant or insect sources. The mammalian enzyme catalyzes

the oxidation of tyrosine and dopa more rapidly than other substrates, whereas the enzyme from non-mammalian sources is usually more effective on substrates other than tyrosine or dopa. Tyrosinase is a member of a group of enzymes, sometimes referred to as the phenolase complex, which first catalyze oxidation of monophenols to diphenols and then dehydrogenation of the diphenols to orthoquinones.[14,15] Orthoquinones are then oxidized and polymerized to a dark pigment.

On the basis of *in vitro* studies it has been possible to elucidate the conversion of tyrosine to melanin according to the steps shown in Figure 1. Here also is illustrated the melanocyte, as it exists in some marine animals, with its pigment granules either clustered in compact masses or dispersed into minute particles.

The influence of hormonal activity on melanin granules will be considered further on. Details of the chemical steps were presented elsewhere.[15,16]

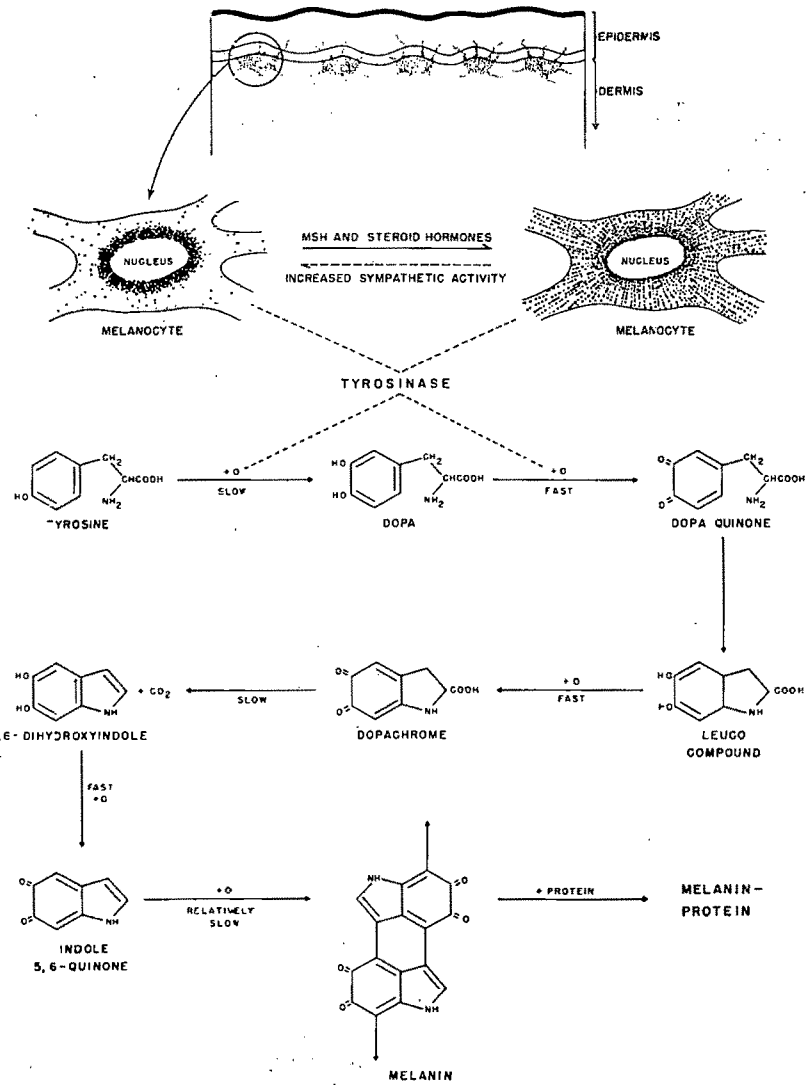


FIG. 1. Enzymic oxidation of tyrosine to melanin.

Recent studies have shown that many of the quinone intermediates in melanin formation, as well as the melanin polymer, are substances which readily react with some amino acids and proteins.[14,17] Consistent with this finding is the fact that melanin in tissues is always attached to protein.[18,19] The stage at which quinones become bound to protein is not known. However, it is possible that melanin in skin, hair and eyes is not

the same because it is formed in the presence of different proteins—proteins which vary according to their respective locations.

The mechanism of the tyrosine-tyrosinase and dopa-tyrosinase reactions has been the subject of great interest and experimentation because knowledge of these reactions is essential to the understanding of the process of melanin formation. The enzymic oxidation of tyrosine to melanin is perplexing for the following reasons. The first oxidation product, dopa, is a necessary catalyst for the oxidation, is more readily oxidized in the presence of tyrosinase and tends to accumulate as the reaction proceeds. One interpretation of the tyrosine-tyrosinase reaction, consistent with known facts, is schematized in Figure 2.[16] Cupric ions of tyrosinase are reduced to the cuprous form by a reversible reaction with dopa. For mammalian tissue this reaction is specific because not even dl-dopa is as effective as l-dopa. Non-specific reducing agents are relatively inactive with respect to mammalian tyrosinase, although quite active on plant tyrosinase. The cuprous tyrosinase-dopa quinone complex is now ready to react with tyrosine. A cuprous tyrosinase-tyrosine complex forms, and dopa quinone is released into the solution. Tyrosine is not required in the formation of dopa quinone but the specifically activated cuprous tyrosinase is needed for combination with tyrosine. The cuprous tyrosinase complex reacts with molecular oxygen to form dopa, but the copper remains in the reduced state. The cuprous ions are then oxidized to cupric ions by oxygen, and the enzyme is returned to its original state. The dopa formed from tyrosine then activates a second tyrosinase molecule which can react with tyrosine.

The dopa-tyrosinase reaction is somewhat simpler since the tyrosine reaction is absent.

The nitrogen of dopa quinone formed in the *in vitro* reactions undergoes ring closure to form the leukocompound, which in turn is oxidized to dopachrome. Dopachrome is decarboxylated to 5,6-dihydroxyindole which undergoes oxidation to indole, 5,6-quinone. The indole quinone is oxidized and polymerized to melanin. Both the leukocompound and 5,6-dihydroxyindole can react with dopa quinone to reform dopa and dopachrome, or indole 5,6-quinone, respectively. By this means dopa tends to accumulate as the reaction proceeds.

Melanin formation consequently depends upon the available concentration of three substances: (1) the enzyme tyrosinase, (2) the substrate tyrosine, and (3) molecular oxygen. The absence of any one of these results in decreased melanin formation. For example, in albinism melanocytes are present in the skin but melanin is not formed because of a genetic absence of tyrosinase in the cytoplasm of the pigment cells.

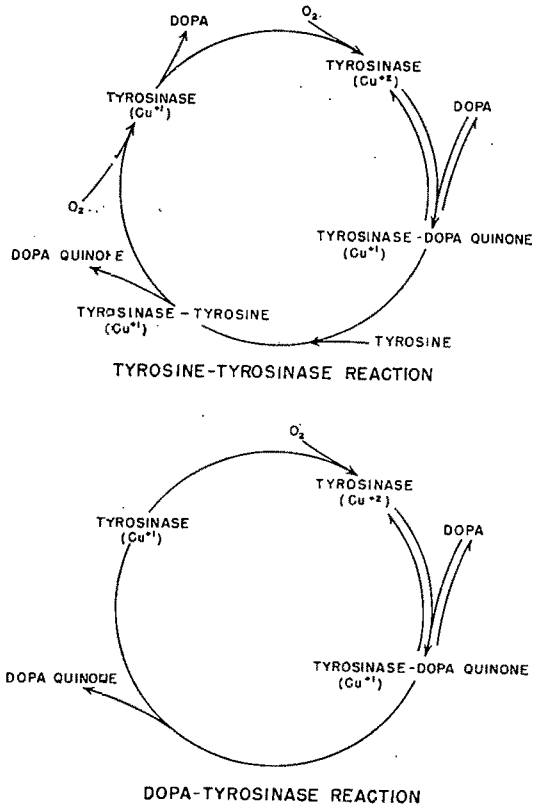


FIG. 2. Reactions of tyrosinase with tyrosine and dopa.

Enzymic control of pigmentation can be correlated with various clinical conditions.[2,15] The reaction of the three basic substances is controlled by several physiochemical factors that determine the rate of melanin formation. Those of hormonal and neurogenic nature will be discussed later. It is sufficient to say at present that temperature, the redox potential within the cell, and agents which bind and inactivate the copper ions of tyrosinase, for example, sulfhydryl groups, are probably the most significant controlling factors. Ionic strength and pH appear to be relatively unimportant.

The temperature of solutions *in vitro* is an important determinant of the reaction rate of mammalian tyrosine-tyrosinase and dopa-tyro-

sinase systems.[20] The reactions proceed relatively slowly at 25°C. but become more rapid at 37°C. Thereafter the reaction rates increase until at about 50°C. when the enzyme is quickly destroyed. Definitive proof of the relationship of temperature to pigmentation *in vivo* is not available. It is reasonable to believe that increased temperature accounts in part for hyperpigmentation of the body folds (palms, axillas, groin, and so on) and mucous membranes seen in normal persons and accentuated in patients with Addison's disease. Exposure of parts of the body to heat for long periods of time, as occurs in repeated use of hot water bottles or sitting before a fireplace or hot stove each day, results in increased pigmentation termed erythema ab igne.

The oxidation-reduction potential near the location of tyrosinase within the melanocyte probably is important in the control of tyrosinase activity because so much of the rate of melanin formation depends upon the valence state of copper and whether or not dopa quinone can be reduced back to dopa. It is possible that ionizing radiations increase melanin formation not only by oxidizing sulfhydryl groups but also by producing a redox potential more favorable to the action of tyrosinase.

In vitro experiments show that sulfhydryl groups can combine with the copper of tyrosinase and inhibit its action.[21] Epidermal extracts contain a diffusible sulfhydryl substance which inhibits tyrosinase *in vitro*.[22] Whether or not this type of inhibition occurs *in vivo* is difficult to ascertain. Available data suggest that it does. For example, one variety of neurospora is dark in color when the organism produces less of a sulfur compound.[23] Also, in mammals, staining technics demonstrate a lack of sulfhydryl groups in the hair bulb in the vicinity of melanocytes.[24] This finding is consistent with the fact that black hair can grow out from white skin. A patient whose hair was predominantly brown, except for a few gray areas, developed increased graying of the hair after each of two courses of BAL therapy for arsenic intoxication.[25] Analyses of light and dark skin of another patient revealed more sulfhydryl groups in the light skin.[26] However, these experiments did not take into account the possibility that the melanin formed in the darker skin may have combined with the sulfhydryl groups, thereby decreasing their concentration.

It has been suggested that oxidation of sulfhydryl groups by ultraviolet light is an important factor in suntanning, that the combination of sulfhydryl groups with arsenic results in hyperpigmentation, and that the destruction of sulfhydryl groups in the skin during inflammatory processes results in postinflammatory pigmentation.[15,22,27]

3. Hormone Control of Pigmentation. An array of pigmentary phenomena in man and animals provides colorful testimony of the importance of hormones in the pigmentation of skin, hair and feathers. For example, skin darkens during pregnancy, in Addison's disease and in some cases of chronic hyperthyroidism. Skin becomes lighter in color in patients with hypopituitarism. Administration of diethylstilbestrol to a hypoovarian female resulted in hyperpigmentation of the nipples and genitalia. The skin of eunuchoid males does not become pigmented after exposure to ultraviolet light unless the patient receives treatment with androgens. Under proper conditions, local application of estrogens and androgens to human beings, guinea pigs and birds produces hyperpigmentation. Estrogens and thyroid extracts modify feather coloring of chickens. Even more spectacular than these observations are experiments showing that removal of the pituitary gland in aquatic animals, such as the frog, results in decreased coloring of the skin whereas injection of pituitary extracts results in marked hyperpigmentation. These findings give added emphasis to the role of hormones in the control of pigmentation.

A. Pituitary hormones: The intermediate lobe of the pituitary gland produces two polypeptides, the melanocyte-stimulating hormones α MSH and β MSH, which can effect increased darkening of melanocytes in man and some animals.[28] (Fig. 3.) These hormones also have been called the melanophore hormone, the melanophore dilating principle, intermedin, and so on. Alpha MSH activity in pituitary extracts is about three times that of beta MSH. Both substances have molecular weights of approximately 4,000 and contain about fifteen different amino acids.[29] As little as 10^{-10} gm. of pure α MSH darkens small pieces of isolated frog skin. This hormone is distinct from other pituitary hormones. However, purified adrenocorticotrophic fractions show small but perceptible MSH activity. The converse is not true; that is, purified MSH possesses no intrinsic adrenocorticotrophic activity. This situation is similar to that of the posterior pituitary hormones, vasopressin and

oxytocin. Vasopressin possesses some intrinsic oxytocin activity although oxytocin has no vasopressin action.

Administration of MSH to human subjects produces hyperpigmentation resembling that seen in Addison's disease.[28] Darkening of the skin in melanin pigmentation. These changes are best observed in dark skin.

Blood and urine MSH can be determined quantitatively by measuring photometrically the degree of darkening of isolated frog skin produced by extracts from these fluids. Urine

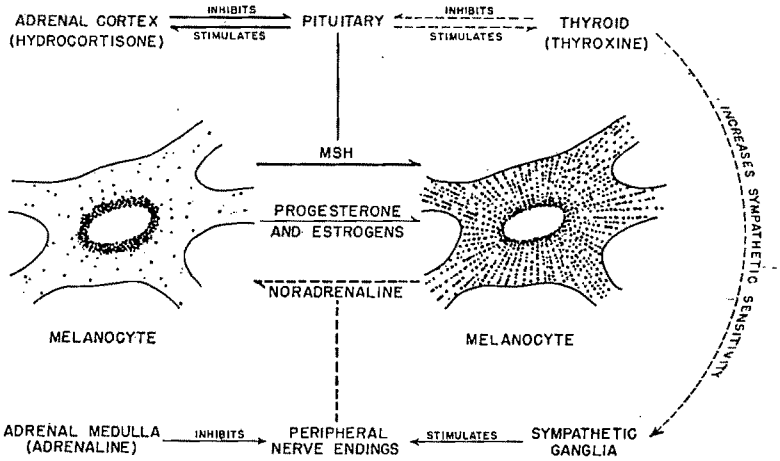


FIG. 3. Hormonal control of pigmentation. Solid lines represent established reactions; dotted lines represent possible reactions; the more prominent reactions are shown by wider lines.

is generalized, although more pronounced on the exposed areas. Pigmented nevi become darker and new nevi form. If large doses of MSH are given skin darkening may be evident within twenty-four hours. The skin returns to its previous color a few weeks after treatment is discontinued.

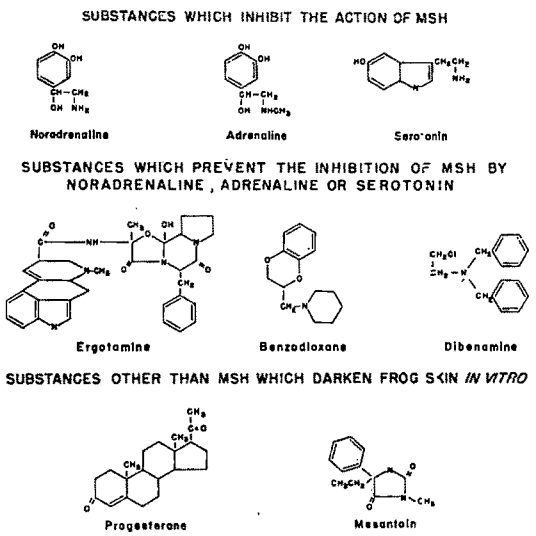
Skin reflectance measurements of white eunuchoid men and ovariectomized women before and after treatment with androgens or estrogens, respectively, showed that darkening of skin following treatment was due primarily to an increase in cutaneous blood flow.[30] Relatively little change in melanin pigmentation was observed. Variations of skin color during the menstrual cycle also were thought to be due primarily to changes in cutaneous blood flow. Some aspects of these experiments should be repeated with a view towards following more closely the changes in melanin pigmentation. Large doses of MSH produce rapid darkening of the skin in man. This effect is much more striking in more pigmented people, such as Negroes, than in fair skinned persons. Variations in skin color in a person under stress, during the menstrual cycle and in conditions of endocrine imbalance might also be due to fluctuations

specimens from normal subjects contain some MSH. The excretion of this hormone is increased during pregnancy, in Addison's disease and in alopecia areata.[31,32] It was increased in the blood of a dog and in rats that had undergone bilateral adrenalectomy.[31,33] MSH was found to be absent from the urine of a hypophysectomized man.[34] MSH excretion is diminished in some patients with panhypopituitarism. The excretion is the same in white, Negro and albino persons. It is not increased in patients with malignant melanoma unless the adrenal cortices are destroyed by the tumor.

Administration of cortisone or hydrocortisone, usually given in 37.5 mg. and 20 mg., respectively, prevents darkening of the skin of bilaterally adrenalectomized human subjects or decreases darkening of skin color in patients with adrenocortical deficiency. Cortisone and hydrocortisone also reduced the levels of MSH to normal in a bilaterally adrenalectomized dog. A patient with severe alopecia areata and markedly increased levels of MSH in the urine excreted normal amounts of MSH after daily oral administration of the following steroids: 37.5 mg. cortisone, 20 mg. hydrocortisone, 5 mg. fluorohydrocortisone. These dosage values were

minimal in that smaller quantities did not produce the same effect.

Darkening of isolated frog skin by MSH can be prevented by adding small quantities of adrenaline® or noradrenaline to the solution.[35] The natural levorotatory forms are more active



than the dextrorotatory isomers. Effective but less active than adrenaline or noradrenaline is serotonin. The inhibition of MSH by these substances can be blocked by sympathomimetic drugs such as ergotamine, dibenamine,® benzodioxane and priscoline.® It is not known whether inhibition of MSH by adrenaline, noradrenaline, serotonin or a related substance has any bearing on normal human pigmentation. However, it is likely that these substances play some part.

B. Steroid hormones: In vitro, progesterone darkens frog skin.[35] Production of progesterone, as well as of MSH, is increased with progression of pregnancy; skin pigmentation follows a similar course. However, local application of progesterone to guinea pig nipples produces no change. Daily application of estrogenic substances to guinea pig nipples produces enlargement and marked darkening of the nipples. Administration of diethylstilbestrol to a hypoovarian female resulted in darkening of the nipples, linea alba and pigmented nevi.[36] The skin of patients with chronic liver disease becomes very dark, presumably because of increased circulating estrogen.[37] Administration of estrogens to a male brown leghorn fowl produces a pigmented feather pattern of the female type.[38] Testosterone has no effect on fowl pigmentation. However, locally applied testosterone induces pigmentation of the bill of the sparrow[39] and the scrotum of the ground squirrel.[40] Eunuchoid males given testosterone are enabled to acquire a good suntan.[41] From these observations it is evident that progesterone, estrogens and androgens alter skin color but the effect varies from one species to another. It is not known whether the blood level of these steroids can affect the release of MSH from the pituitary gland. As mentioned previously, cortisone and hydrocortisone influence pigmentation, not directly, but at the pituitary level by inhibiting the formation or release of MSH.

C. Thyroid hormones: Thyroxine is required for normal pigmentation of the feathers of male and female brown leghorn fowls.[38] Injection of crude thyroid extract into rainbow trout blanches the skin.[42] The active agent is not thyroxine, diiodotyrosine or an inorganic iodide compound. There is a clinical impression that patients with hyperthyroidism tend to have vitiligo, although it is uncommon for patients with vitiligo to have hyperthyroidism. One patient with hyperthyroidism and vitiligo experienced complete repigmentation following thyroidectomy.[43]

From these observations it is apparent that the thyroid gland plays a part in pigmentation. However, its exact status is not established. Little is known concerning the relationship of the thyroid gland to the output of MSH by the pituitary gland.

D. Other hormones: It has been claimed that the pars tuberalis contains a substance which exerts an opposite effect from that of MSH on the toad and eel.[44,45]

Two drugs, mesantoin and chloroquin, recently were shown to affect pigmentation. Although their mode of action is not known to be hormonal, they will be mentioned briefly. Prolonged administration of mesantoin, an anticonvulsant, has been associated with hyperpigmentation resembling that of Addison's disease.[46] This drug has an MSH-like action on isolated frog skin.[35] It is possible that it can exert an MSH-like action in man also. Chloroquin has lightened the hair color of some children about four weeks after its administration for treatment of lupus erythematosus.[47,48] Whether these drugs produce a change in pigmentation through a hormonal, neurogenic or enzymic process has not been established.

E. Mechanism of hormone action: A most important problem is determining the action of

hormones on melanocytes. Through what mechanism do MSH and progesterone darken melanocytes, and how do substances like adrenaline, noradrenaline and serotonin inhibit MSH activity? The effect of MSH on frog melanocytes is rapidly reversible. Under proper conditions *in vitro* the cells may darken in about fifteen minutes and lighten again in a similar period of time. Darkening is brought about by dispersion of the conglomerate masses of melanin pigment granules. Relatively little change occurs in the over-all size and shape of the melanocyte during this process.[49] Oxygen is required for optimum darkening but not for the lightening process. MSH does not darken melanocytes in a stream of hydrogen. Agents that block sulfhydryl groups inhibit the action of MSH, although in themselves they can darken melanocytes. Melanocytes placed in solutions of low pH or ionic strength deepen in color. The physical change inside the cell induced by MSH is closely related to the biochemical integrity of the melanocyte. This phenomenon is similar to that of muscular contraction, a physical change which nonetheless is closely related to the biochemical reactions occurring in the muscle fiber.

The variations in the melanocyte which take place shortly after addition of MSH are probably different from those observed after the cell has been kept in a darkened state for long periods. In the latter case there is probably an increase in new melanin formation. While small amounts of MSH effect a reversible darkening of frog melanocytes *in vivo* or *in vitro*, large amounts produce permanent darkening.

4. Neurogenic Control of Pigmentation. Melanocytes are derived from the neural crest and resemble nerve cells in several respects. They are dendritic in shape, produce dopa which is similar in chemical structure to noradrenaline produced by some nerve tissue, and give rise to neoplasms which do not respond to x-ray therapy any more favorably than do most nerve cell tumors. It is therefore not unexpected that melanocytes should be subject to some type of neurogenic control.

In fish, sectioning the sympathetic nerve to a fin results in darkening of that fin. As the nerve regenerates, the fin returns to its normal color. On the other hand, faradic stimulation of the intact nerve causes blanching of the fin.

Examples of neurogenic factors in human pigmentation are numerous. In 90 per cent of patients with neurofibromatosis pigmented macules or café au lait spots are present. The exact etiology of these lesions is not known but it is interesting that they appear in persons with nerve lesions.

There is some evidence to support the view that vitiligo is caused by increased activity of the sympathetic nervous system at the peripheral nerve endings. A patient with vitiligo and transverse myelitis had no depigmentation below the level of paralysis, whereas usually in vitiligo depigmentation is observed on the lower portions of the body. Another patient developed vitiligo several years after having had poliomyelitis. There was less depigmentation on the leg having nerve damage.

Opposed to the observations in vitiligo is the finding that following cervical sympathectomy and production of the Horner syndrome the iris of the involved eye either remains unchanged or becomes lighter in color. As mentioned earlier, the melanocytes of the eye may respond to different neurogenic control than do those in the skin.

The view is held among ophthalmologists that melanomas of the eye arise primarily from nerve cells of the sheath of Schwann where the nerve fibers traverse the uveal tract.

In addition to these clinical observations are the previously discussed *in vitro* findings that two hormones, adrenaline and noradrenaline, both produced by nerve tissue, can inhibit MSH action and cause blanching of frog melanocytes.

At the present time a moderate amount of information is available concerning the cytology of melanocytes and the control of pigmentation by enzymic and hormonal factors. It is hoped that our knowledge of neurogenic control will be similarly expanded.

NORMAL PIGMENTATION

In normal persons the color of skin, eyes and hair may vary from near white to black. These differences in pigmentation are not due to variations in the number of melanocytes but rather to the pigment producing capacity of the melanocytes in a given subject. The amount of pigment in a particular melanocyte is determined by the balance of enzymic, hormonal and neurogenic factors. The albino, white man and Negro have approximately the same number of melanocytes in any particular location. Equal quantities of urinary MSH are excreted. But in the albino there is a genetic absence of

tyrosinase so that melanin cannot be formed. It is possible that the darker skin coloring of some races may be explained in one or more of the following ways: (1) The total amount of tyrosinase available for melanin formation may be increased. (2) The normal inhibitors of tyrosinase may be decreased. (3) An oxidation-reduction potential in the melanocyte may be more favorable for tyrosinase action.

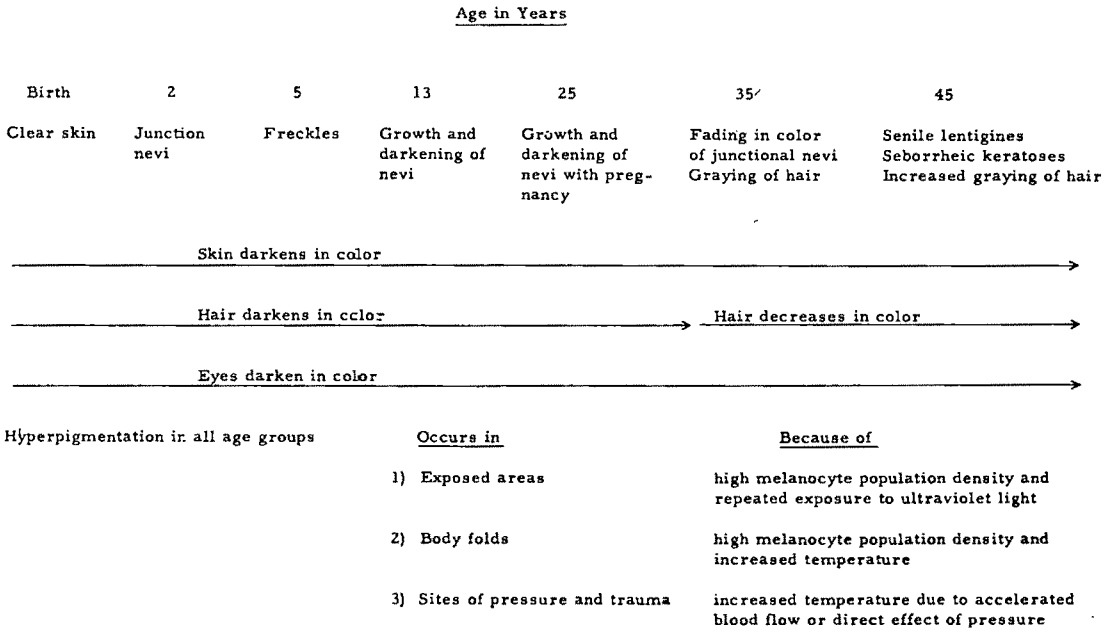


FIG. 4. Normal pigmentation life chart.

At birth the skin is usually free from pigmentary lesions. Only 2.7 per cent of white infants, but 15.6 per cent of Negro infants, were reported to have pigmented lesions at birth.[50] Sixty per cent of Negro infants and 0.5 per cent of white infants have sacral or mongolian spots. These blue-colored areas represent diffuse collections of pigmented melanocytes in the dermis. Usually they cease to be visible after a few years.

With growth and aging, the skin, eyes and hair become darker. (Fig. 4.) This increased darkening is especially notable between birth and puberty in persons of fair or medium dark coloring. It is common for a child with almost snow-white hair in infancy to develop dark brown hair by the time puberty is reached. Hair frequently darkens following a severe illness. Late in life hair becomes gray. The infant who is blue eyed at birth may become brown eyed at a few months of age. Afterwards the eyes continue to darken, and the rate of darkening is greater in the female. Negroes are often light in color at birth but become much darker within two weeks. Their eyes undergo rapid change from light brown to dark brown or black.

At about two years of age pinpoint-sized, flat, pigmented spots appear, usually on the upper portions of the body. They frequently follow stress reactions, such as a severe cold or one of the infectious diseases of childhood. These minute lesions are junctional nevi which develop as a result of increased melanocyte activity at the epidermal-dermal junction. In very light complexioned children or in albinos the nevi are flesh colored. They usually increase in size and color with increasing growth and development of the child and with the advent of new illnesses. It is possible that MSH released from the pituitary gland during periods of stress is responsible for the nevi.

Although nevi will be discussed in detail later, some aspects will be considered now. According to textbook connotation, there are an average of twenty nevi per person, although they may vary from one to a hundred. However, no data have been given to support this impression. Nevi counts should be done in both sexes, at different ages and for different types of skin coloring. An early nevus may follow one of several courses. It may remain unchanged in size, it may increase or regress. Usually all these changes occur but at

different periods; that is, after the initial appearance of the nevus, it may remain stationary until a new illness develops or until puberty is reached. It then darkens and grows. However, later, at about the age of thirty, the nevus may show decreased melanocyte activity and regress. At the time the nevus enlarges it may develop in other ways as well. Although it may become merely a larger junctional nevus, a compound nevus results if some melanocytes move into the dermis while others remain at the basal layer. A dermal nevus forms if all the melanocytes are limited to the dermis proper.

At five to seven years of age some persons develop freckles on areas exposed to sunlight. Freckles are prominent from their onset until about the age of twenty when they become less apparent.

During puberty nevi darken and grow and new ones appear. A similar course is noted in pregnancy.

During the thirties graying of hair may become evident. In the forties new nevi cease to appear but senile lentigines, popularly known as liver spots, and seborrheic keratoses may form. Senile lentigines will be discussed later. Seborrheic keratoses are hyperkeratotic, greasy-surfaced lesions which are often very dark. Their color is in part due to melanin.

In normal persons pigmentation is not uniform over the entire body. Hyperpigmentation occurs on all exposed areas, such as the face, neck and hands, in the body folds, such as the axillas, groin and palms, and at the sites of pressure and trauma, such as the location of cuts, burns or the wrist band area. A darkening of these regions may result from one or more of the following conditions: (1) high melanocyte population density, (2) repeated exposure to ultraviolet light, or (3) increased temperature due to accelerated blood flow or direct effect of pressure.

Those areas that are normally hyperpigmented are labile insofar as color is concerned. In diseases with a tendency towards hyperpigmentation, Addison's disease for example, the sites of physiologic hyperpigmentation become darker. In vitiligo the same areas become lighter. These observations are so constant that from them a law of skin pigmentation can be formulated: Normal areas of hyperpigmentation are predisposed to change either in the direction of more hyperpigmentation or depigmentation.

VARIATIONS IN MELANIN PIGMENTATION

A classification of variations in skin pigmentation is outlined in Table 1. The list is made up of entities which either are common or else illustrate a basic process pertaining to pigmentation. It is worth while pointing out the reasons for using this type of classification. In the past, efforts were made to divide pigment abnormalities into two main groups based upon either local and systemic factors or those of external and internal origin. Such schemes are confusing because of much overlapping in the two groups. In Table 1, nine different etiologic groups are divided according to hyperpigmentation or hypopigmentation. Here, too, overlapping of the etiologic groups occurs; for example, although the ability to freckle is genetically transmitted, the physical factor of ultraviolet light is a necessity for their development. The tendency to form junctional nevi may be genetic in part but also is intimately related to hormonal factors. Vitiligo may be placed in either the genetic or neurogenic groups. Other examples of overlapping will be apparent. In spite of these limitations the classification is easy to understand and simple to use. Each entity will be discussed briefly.

1. Genetic Factors. The variations in pigmentation from one person or race to another were discussed in the section on normal pigmentation.

Freckles or ephelides (Fig. 5A) are associated with a single dominant gene which is linked with that for red hair. Both genes are associated with the same chromosome. A high correlation exists between freckles and red hair but a low one between freckles and eye color. Freckles are round or irregularly shaped flat spots which are light brown in color. They vary in size and occur chiefly on exposed areas such as the face, neck, upper back, shoulders and backs of hands. The lesions appear initially at about five years of age and develop after exposure to sunlight. Freckles darken during the summer and lighten in winter. Their color fades in adult life. Microscopically a freckle shows only hyperpigmentation of melanocytes at the epidermal-dermal border.

Pigmented nevi can be divided into four groups depending upon the site of melanocyte proliferation: Junctional nevi or lentigines (Fig. 5B) arise from proliferation of melanocytes at the epidermal-dermal junction. They are usually brown to black hairless macules. They

are by far the commonest type of nevus and can be produced by injections of MSH. Compound nevi are composed of melanocytes at the epidermal-dermal junction and in the dermis. They vary from colorless to black, may be flat or raised and may or may not contain hair. Their rate of occurrence is second to junctional nevi. Dermal nevi consist of melanocytes grouped in the dermis. Clinically they resemble compound nevi but occur less frequently. Blue nevi are a form of dermal nevi which usually occur in the lower two-thirds of the dermis. The color varies from blue to black depending upon the quantity and location of melanin in the dermis. They are seen most frequently in persons belonging to the Oriental races.

Most nevi appear after birth, but some large pigmented ones are present at birth. (Figs 6A and B.) They probably are a result of abnormal migration of melanocytes from the neural crest to the skin. The factors controlling this migration are not known. These nevi may be only a few millimeters in size or large enough to cover most of the body. The elements of junction, compound and dermal nevi may all occur in one lesion. Occasionally malignant change has been reported. Some women who have large nevi believe they darken at menstruation.

The development of nevi with age was discussed earlier. Most nevi are usually new growths, and there is no evidence that their

TABLE I

VARIATIONS IN MELANIN PIGMENTATION

Etiologic Factors	Hyperpigmentation	Hypopigmentation
Genetic	Racial factors Freckles (ephelides) Pigmented nevi Senile lentigines	Albinism (partial or total) Premature graying of hair
Physical	Ionizing radiations Heat	Severe thermal burns Severe trauma
Chemical	Heavy metal intoxication Photosensitization (psoralens, tar)	Hydroquinone derivatives Chemical burns
Endocrine	Addison's disease ACTH therapy Hyperthyroidism Acromegaly Pregnancy Estrogen or androgen therapy Chronic hepatic insufficiency	Addison's disease Panhypopituitarism Hyperthyroidism
Neurogenic	Café au lait spot Lines of demarcation	Vitiligo Alopecia areata
Nutritional	Chronic malnutrition Niacin deficiency (pellagra)	Pantothenic acid deficiency Para-aminobenzoic acid deficiency
Infectious and inflammatory	Chronic systemic infections (e.g., TBC) Chronic dermatoses	Leprosy Pinta
Neoplastic	Chronic illness with neoplasm Acanthosis nigricans Melanoma	
Unclassified	Scleroderma Hemochromatosis Skin graft Polyostotic fibrous dysplasia Intestinal polyposis	Scleroderma Skin graft



5A 5B

FIG. 5. A, freckles; lighten in winter and darken in summer B, junction nevi or lentigines; not present at birth; very common.

origin is from congenital misplaced cells which hitherto were unrecognized. It is possible that because of local anatomic variations such as blood supply MSH, produced by a stress response, stimulates the formation of new nevi. However, blue nevi may represent collections of melanocytes which in their embryonic development failed to reach the epidermal-dermal junction.

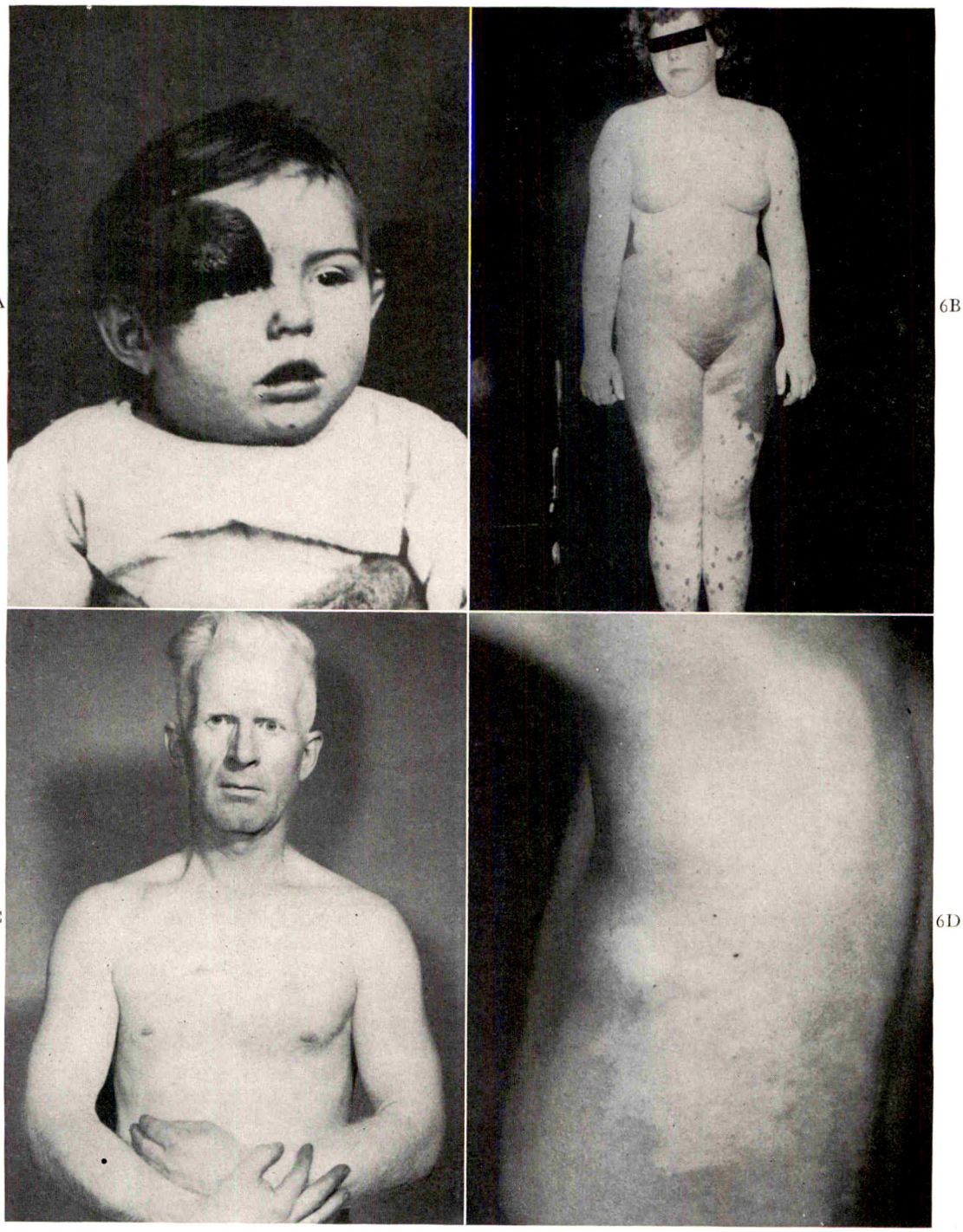
While the tendency to form nevi is partially under genetic control, MSH is an important contributing factor.

Senile lentigines are yellow to brown, vary in size and may be as large as a centimeter in diameter. They usually occur in adults in areas repeatedly exposed to sunlight, such as the hands and face. The layman refers to senile lentigines as "liver spots" because their color resembles that of fresh liver. Microscopically they are similar to lentigines or junctional nevi. However, they occur in people with a genetic tendency to their formation after the skin has been exposed to sunlight for many years.

Albinism in the experimental animal is transmitted as a non-sex linked, simple Mendelian recessive factor. Albinism is present from birth. It may be divided into complete, incomplete and partial types. Pigment does not form because of genetic absence of tyrosinase in the melanocyte. (Fig. 6C and 6D.)

In cases of premature graying of hair, as in vitiligo, the age of onset tends to follow a genetic pattern. However, additional factors, particularly neurogenic, are probably involved. The pigment law, if carried to the extreme, might permit the conclusion that formation of gray hair in normal persons is a vitiliginous process, for hair, being highly pigmented, would be likely to undergo early depigmentation.

2. *Physical Factors.* Ionizing radiations, such as ultraviolet light, alpha rays and x-rays, stimulate formation of new melanin and also darkening of pre-existing melanin.[15] As mentioned earlier, oxidation of sulfhydryl groups by ionizing radiations might make tyrosinase more available for melanin formation. Also one or more of the following factors may play a role: (1) The radiations may promote conversion of tyrosine to dopa so that the latter then can catalyze the tyrosine-tyrosinase reaction. (2) The oxidation-reduction potential in the melanocyte may undergo change so that tyrosinase activity is increased. (3) In the case of sunlight, temperature may be increased, allowing for acceleration in the rate of melanin formation. In addition to these three factors, melanin already



6A 6B

6C 6D

FIG. 6. A, large nevus on face present since birth; relatively rare. B, large bathing trunk nevus present since birth; relatively rare. C, complete albinism. D, partial albinism in a seven year old boy; hypopigmentation present since birth.

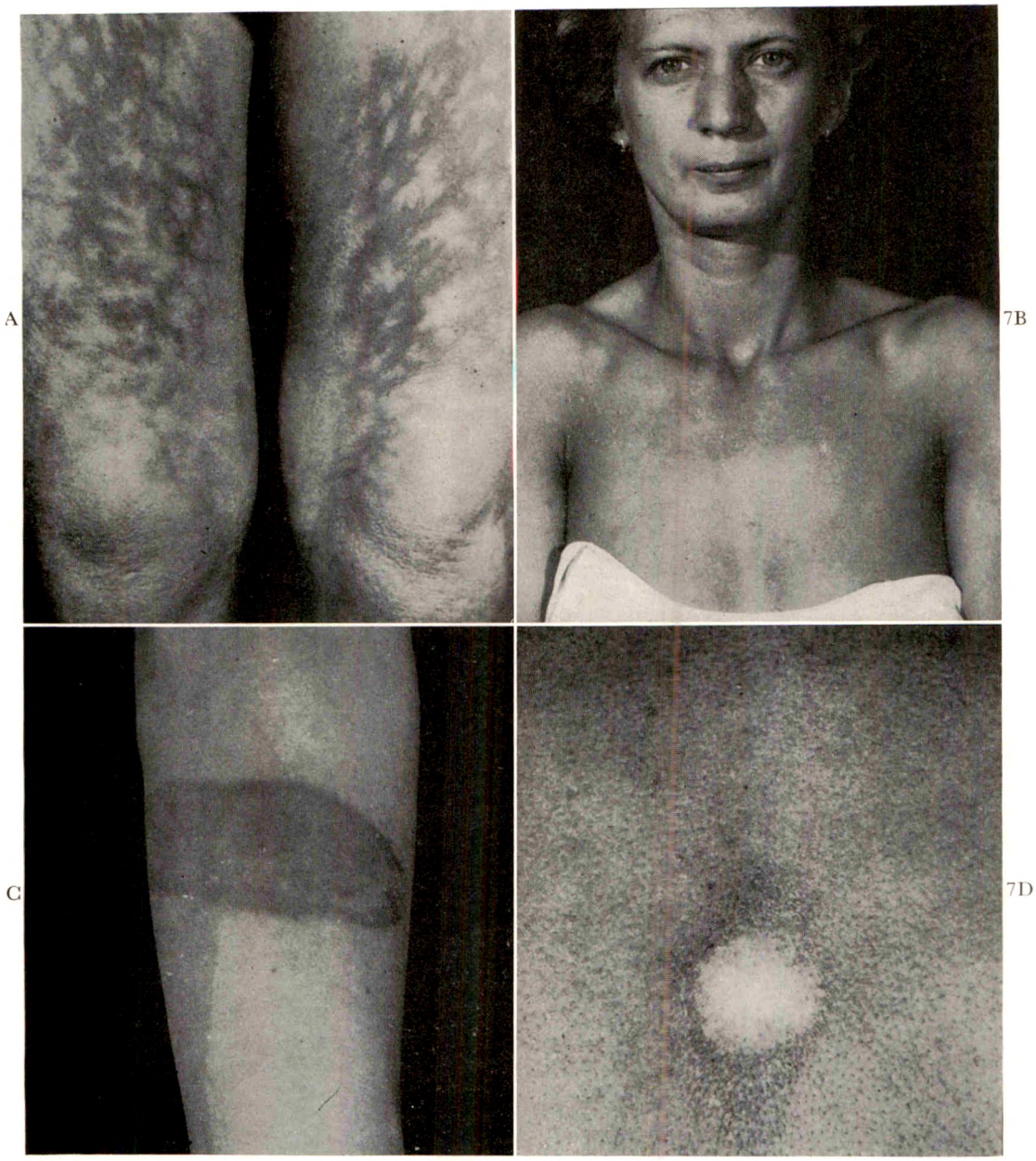


FIG. 7. A, erythema ab igne of thighs; reticulated hyperpigmentation from prolonged use of heating pad. B, hyperpigmentation following arsenic intoxication; note "raindrop" appearance of thoracic pigment. C, marked hyperpigmentation following application of 8-methoxypsoralen and subsequent exposure to sunlight. D, localized depigmentation of Negro's back after four weeks' continuous application of an ointment containing 20 per cent monobenzylether of hydroquinone.

resent may become darker because of oxidaon to a darker form.

In most instances a suntan or other type of adiation-induced hyperpigmentation fades a :w days or weeks after the provocative stimulus, nd the skin returns to its previous shade. Howver, this sequence is not invariable. Sometimes a severe sunburn is followed by large freckles which remain permanent. Intensive exposure to roentgen rays may produce lasting hyperpigmentation. Why this hyperpigmentation should persist is not known. Perhaps the pigment formed under this profound stimulus is permanently dispersed in the melanocyte, much in

the manner of the irreversible darkening produced in frog melanocytes by administration of huge amounts of MSH.

Years ago reticulated hyperpigmentation, or erythema ab igne, was common on the legs of people who regularly exposed themselves to the heat of a fireplace or stove. (Fig. 7A.) Today this type of pigmentation is less common but it still occurs in persons who use hot water bottles for long periods of time and in workers who are frequently in proximity to hot furnaces. Hyperpigmentation probably results from a direct increase in temperature, as well as an indirect one through heightened vascularity, which accelerates the tyrosine-tyrosinase reaction.

Thermal burns or trauma to the skin severe enough to destroy melanocytes results in depigmentation. Like most nerve cells, melanocytes regenerate slowly.

3. Chemical Factors. Administration of arsenic, silver or bismuth compounds, may produce hyperpigmentation. Arsenic hyperpigmentation characteristically assumes a "raindrop" pattern on the exposed areas and trunk. (Fig. 7B.) Small, relatively pale spots are scattered throughout the areas of hyperpigmentation. Arsenic hyperpigmentation may be difficult to distinguish from that seen in Addison's disease. Arsenic keratoses may occur, especially on the palms and soles.

Patients with silver (argyria) or bismuth intoxication develop slate gray or blue hyperpigmentation on the exposed areas which imparts a cyanotic appearance. Although the increased pigmentation is seen only on areas exposed to sunlight, the metal also is deposited in the skin of the covered regions.

The hyperpigmentation from arsenic may be due to inactivation of sulfhydryl groups, making copper more available for tyrosinase action. Most of the hyperpigmentation from silver and bismuth is not due to melanin but represents deposits of the metallic metal in the skin, especially in the sweat glands.

In photosensitization, several substances, particularly the psoralen derivatives present in many essential oils, increase pigmentation when applied to the skin or taken orally prior to ultraviolet light exposure. (Fig. 7C.) The mechanism of this process is unknown. 8-Methoxypsoralen can be used to treat some patients with early and limited vitiligo.[8]

In the case of hydroquinone derivatives, depigmentation occurs in some persons following application of monobenzylether of hydroquinone. (Fig. 7D.) Evidence is present that the tyrosine-tyrosinase reaction is inhibited by hydroquinone formed from the monobenzylether of hydroquinone.[51]

Chemical burns may cause depigmentation resulting from destruction of melanocytes.

4. Endocrine Factors. Patients with adrenocortical insufficiency (Addison's disease) frequently show hyperpigmentation of the exposed areas, body folds, sites of pressure and trauma, and of the mucous membranes. (Fig. 8A and 8B.) Nevi darken and new nevi form. Occasionally these patients develop vitiligo with depigmentation in these areas, but with marked hyperpigmentation of the rest of the skin.

Increased release of MSH from the pituitary gland occurs following decreased adrenocortical function. MSH markedly accentuates pigmentation in those areas where hyperpigmentation normally occurs and may induce formation of new nevi.

The depigmentation in some patients with adrenal insufficiency may be explained by assuming that extensive destruction of the adrenal glands evokes a compensatory increase in sympathetic activity resulting in vitiligo. In these patients the hyperpigmenting and depigmenting processes proceed simultaneously.

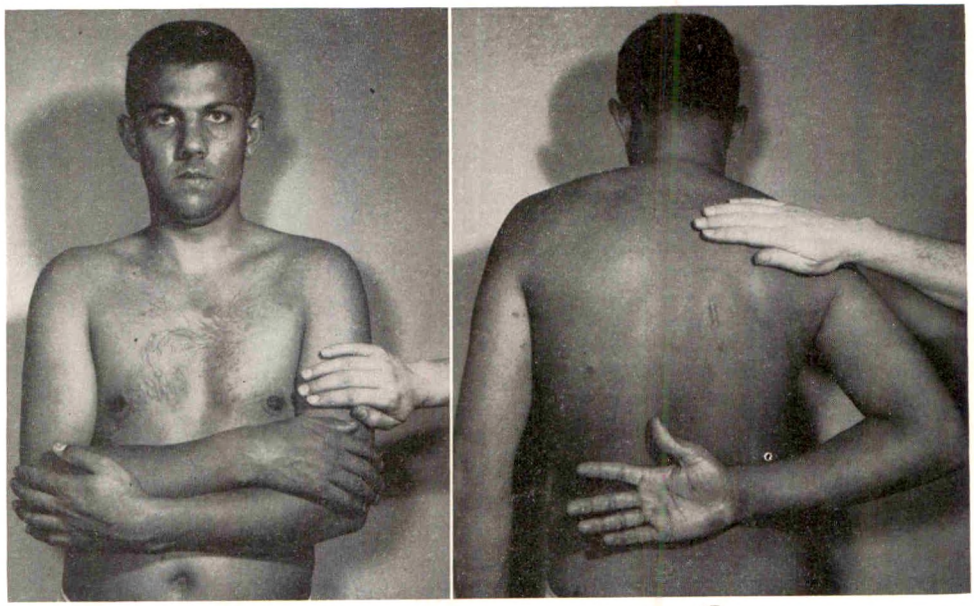
Prolonged administration of ACTH may produce hyperpigmentation because of MSH present as a contaminant in the ACTH preparation.

It is frequently remarked that patients with early hyperthyroidism tend to have vitiligo but no statistical study on this point apparently has been made. Hyperpigmentation sometimes occurs in severe, chronic hyperthyroidism, possibly because of increased release of MSH from the pituitary gland.

Hyperpigmentation when present in acromegaly may be due to MSH.

Hyperpigmentation (melasma*) develops over the face, usually in a blotchy fashion, constituting the "mask" of pregnancy. The nipples, genitalia and linea alba darken. Nevi may deepen in color and increase in size. Several hormones which affect melanocytes increase in the blood and urine during pregnancy. The

* In this paper the term melasma refers to the hyperpigmentation occurring usually on the exposed areas, body folds or sites of trauma associated with increased urinary excretion of MSH.[52] The brown discoloration which occurs during pregnancy is due to melasma rather than to any green pigment. It is more correctly designated melasma then chloasma.



8A 8B

FIG. 8. A, anterior view of twenty-seven year old man with Addison's disease of seven years' duration; compare color with hand of normal subject selected by patient as representing his complexion prior to illness. B, back of patient shown in Figure 8 A; note hyperpigmentation of palmar creases and scars on back.

levels of MSH, progesterone and estrogens rise with progression of pregnancy. Within five days after delivery MSH levels return to within normal limits. It is possible that all of these factors contribute to melanocyte darkening. In most patients pigmentation fades a few months after delivery; however, pigmentation sometimes is permanent.

In white persons with estrogenic or androgenic deficiency the skin has a pallor due largely to decreased cutaneous blood flow. Administration of the deficient hormones corrects this situation. The hormones also may affect melanin pigmentation, particularly in the darker subjects.

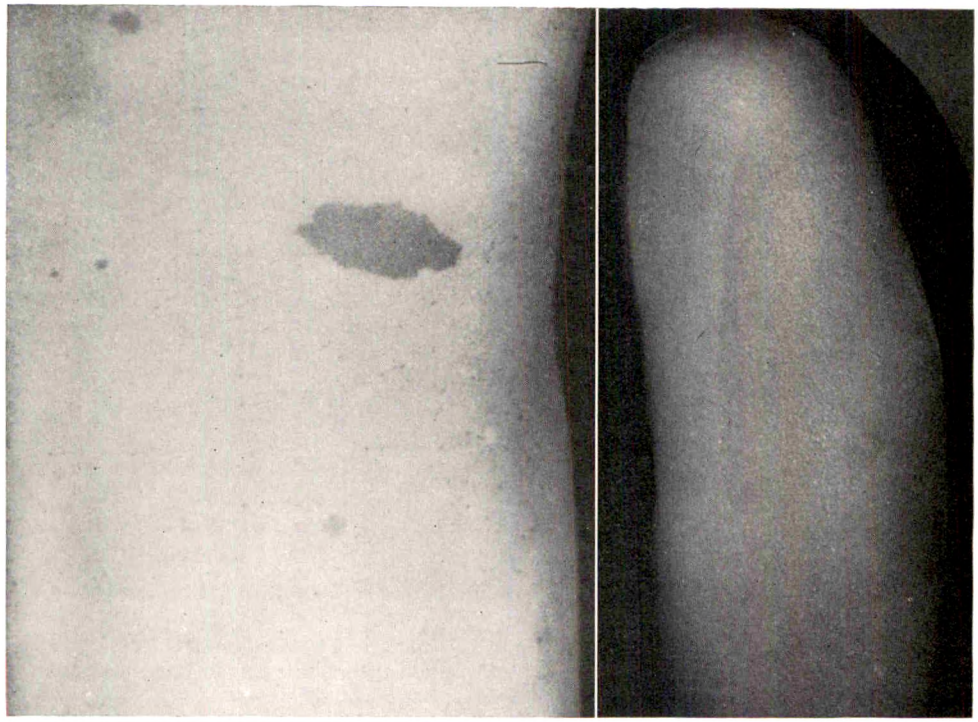
Some patients with chronic hepatic insufficiency become very dark. It has been suggested that increased circulating estrogens are responsible for this hyperpigmentation.[37]

In panhypopituitarism the skin is light because circulating MSH is decreased as a result of reduced pituitary function.

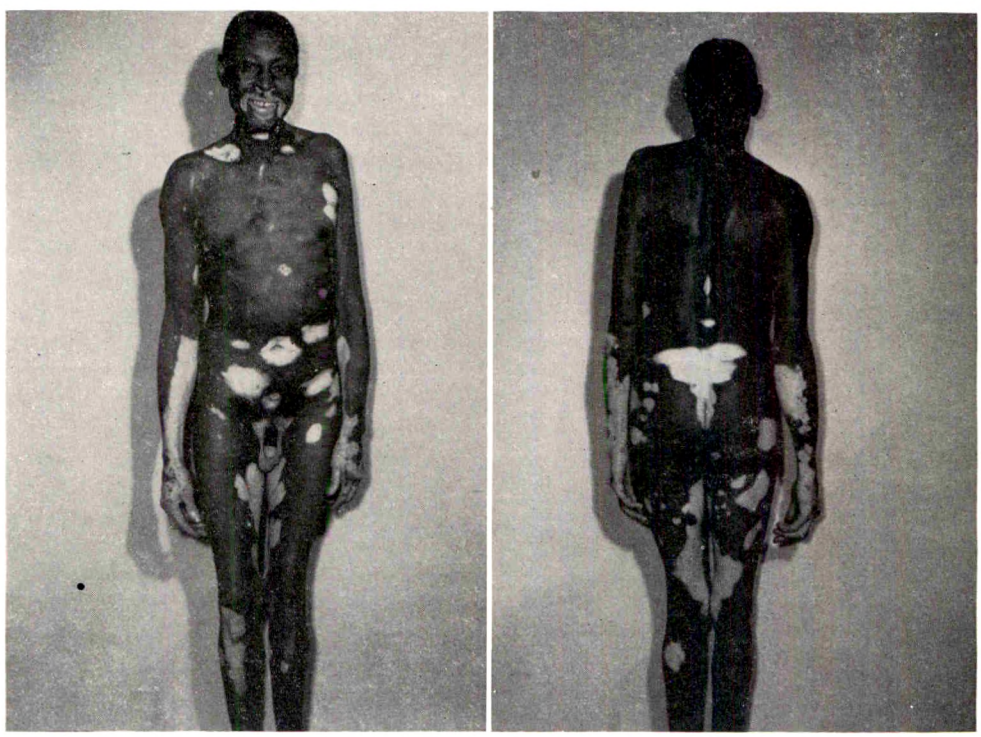
5. Neurogenic Factors. Café au lait spots (Fig. 9A) are hyperpigmented macules of variable size and shape which usually appear after birth. Approximately 10 per cent of normal persons and 90 per cent of patients with neurofibromatosis have café au lait spots.[53,54] However, only 0.2 per cent of normal persons have three or more whereas about 75 per cent of those with neurofibromatosis have this number. Microscopically café au lait spots show hyperpigmentation of the basal layer and are indistinguishable from the ordinary freckle. These lesions are grouped with factors under neurogenic control because patients with neurofibromatosis have nerve lesions and neural control of melanocyte function may be affected.

In people with more pigmented skin, such as the Japanese, the arms and chest of some individuals show sharply divided areas of pigmentation.[55,56] These lines of demarcation are fairly common in Negroes. (Fig. 9B.) Along the anterior aspect of the arm a sharp border may separate a lateral dark portion from a medial lighter area. It has been suggested that this sharp division of color depends upon neurogenic factors.

In vitiligo hypopigmentation occurs, usually in one or more of the following areas: hands, arms, face, neck, axillas and inguinal regions.[57] (Fig. 9C and 9D.) Approximately 1 per cent of the total population of this country has vitiligo. In 50 per cent of the cases hypopigmentation develops before the age of twenty. Repigmentation to some degree, although not complete, occurs in 50 per cent of patients with vitiligo at some time in the course of their illness, usually in the summer following exposure to sunlight. In



9A 9B



9C 9D

FIG. 9. A, café au lait spots. B, line of demarcation on Negro's arm; common. C, vitiligo; note involvement of common sites, face, arms, umbilicus and genitalia. D, posterior view of patient shown in Figure 9 C.

50 per cent of the cases relatives are found to have vitiligo. The inherited tendency toward development of vitiligo is expressed as a dominant factor. The hypopigmentation usually becomes more extensive following severe stress such as an acute illness or operation. Although the etiology of vitiligo has not been determined, the following points suggest that hypopigmentation may result from increased activity of the sympathetic nervous system at the peripheral nerve endings near the melanocytes: (1) L-noradrenaline tested on isolated frog skin was found to be a specific inhibitor of MSH. Frog melanocytes became light in color upon addition of noradrenaline.[35] (2) In some fish sectioning of a fin nerve results in darkening of skin distal to the section. Upon regrowth of the nerve the skin becomes light again. Faradic stimulation of the intact nerve produces a decrease in color. A patient with transverse myelitis of thirteen years duration developed vitiligo four years ago and no depigmentation has occurred below the level of paralysis.[57] (3) Vitiligo often occurs in segments corresponding to nerve distribution.[57–59] (4) Under resting conditions, more sweat occurs in areas of vitiligo than on adjacent pigmented skin.[57] (5) A report on the use of the capillary microscope *in vivo* indicates that blood vessels are constricted in patches of vitiligo.[60]

If a patient with vitiligo becomes jaundiced or develops yellow skin from ingestion of atabrine only the non-vitiliginous areas become yellow. The pigmentation due to bile or atabrine is not seen in the areas of melanin hypopigmentation.[88,89] Studies have not been made on the affinity of melanin or melanocytes for these other pigments.

Patients with diffuse patches of baldness (alopecia areata) and increased emotional tension frequently exhibit gray hair and vitiligo. It is possible that hypopigmentation of hair and skin is due to increased activity of the sympathetic nervous system.

6. Nutritional Factors. A few weeks on a starvation diet may produce melasma of the face.[15] If starvation is continued the hyperpigmentation may become generalized. It is possible but not proved that increased pituitary activity increases the release of MSH so that hyperpigmentation results.

In pellagra, hyperpigmentation of the exposed areas is common following subsidence of the acute dermatitis. The darkening represents the dual processes of photosensitization and post-inflammatory pigment deposition. Reduction in sulfhydryl groups following the acute reaction may be responsible for the hyperpigmentation.

Pantothenic and paraaminobenzoic acids are known to be required for normal pigmentation of the hair of some animals but not that of man. Administration of large doses of paraaminobenzoic acid to man produces repigmentation of gray hair in some subjects.[61]

7. Infectious and Inflammatory Factors. Chronic infections or almost any severe illness of long duration may be associated with development of melasma. As in other instances of melasma, it may be of endocrine etiology.

Hyperpigmentation accompanies prolonged rubbing and scratching of the skin indulged in by patients with chronic dermatoses such as severe atopic dermatitis or postinflammatory lesions. (Fig. 10A.) Increased temperature and destruction of sulfhydryl groups may increase melanin formation.

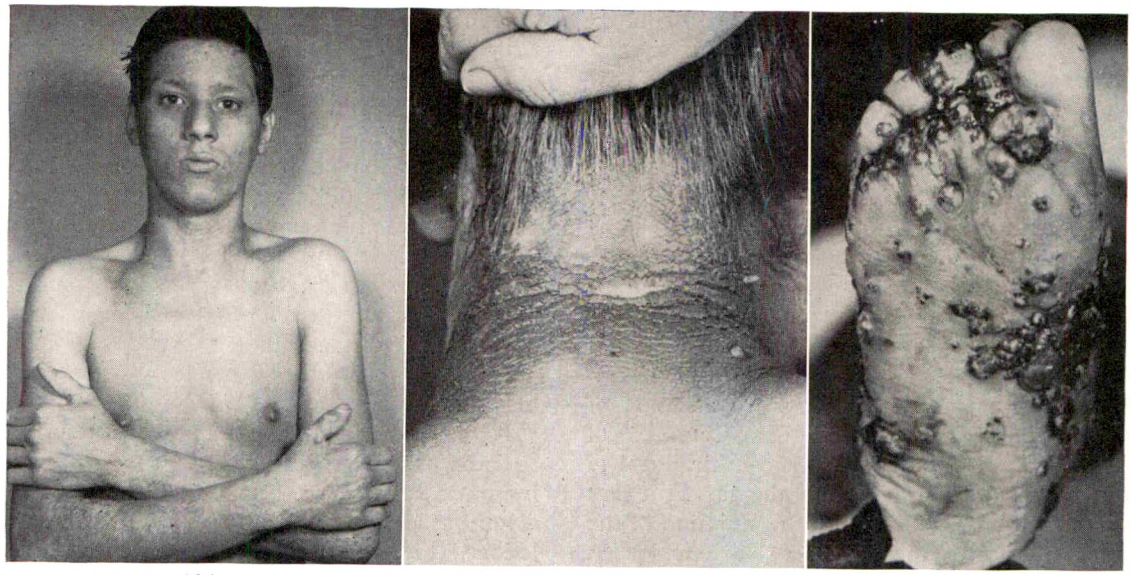
Hypopigmented macules are common in leprosy, especially in the tuberculoid type.[62] In such cases skin lesions are co-extensive with nerve involvement. The cause of hypopigmentation is not known; it may be neurogenic.

Hyperpigmentation is seen early in the course of pinta. However, after the disease has been present for a few years, extensive depigmentation may develop.

8. Neoplastic. As mentioned previously, severe chronic illness with neoplasm often is associated with hyperpigmentation.

In acanthosis nigricans, pigmented verrucous excrescences occur in the axillas and other body folds.[63] (Fig. 10B.) Acanthosis nigricans is divided into benign and malignant types. Although the dermatosis itself is benign, the so-called malignant type is associated with adenocarcinoma, particularly of abdominal organs. Benign acanthosis nigricans begins at birth, in childhood or at puberty. The malignant type may begin at any age and is preceded, accompanied or followed by an adenocarcinoma. The dermatologic findings are the same in both types. The relationship of these changes to malignancy has not been elucidated.

A few patients with melanoma develop generalized dark blue hyperpigmentation resembling that of argyria.[64] This coloring represents melanin in the dermis which appears blue when viewed from the surface of the skin. The tumor produces large amounts of 5,6-dihydroxyindole,



10A 10B 10C

FIG. 10. A, hyperpigmentation in a seventeen year old boy with chronic atopic dermatitis. B, malignant acanthosis nigricans in a twenty-eight year old woman. C, melanoma.

an unstable substance which is rapidly oxidized, even in the absence of tyrosinase, to melanin.

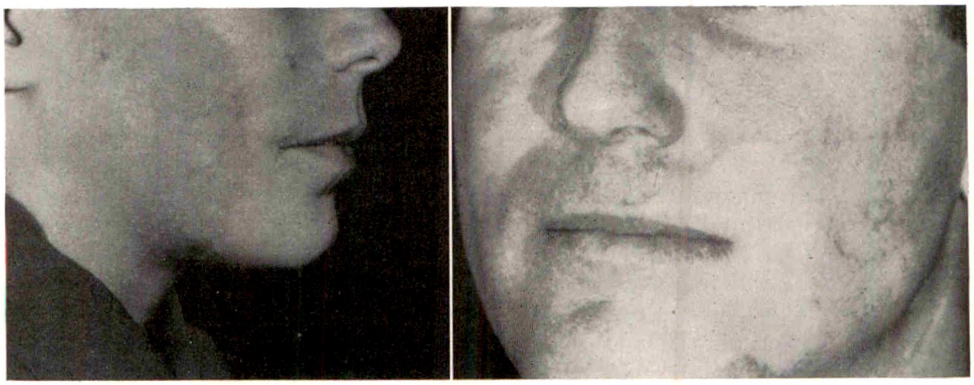
9. Unclassified Factors. Both hyperpigmentation and hypopigmentation occur in patients with scleroderma. The reasons for these changes remain obscure.

The skin discoloration in hemochromatosis is characteristically bronze. Often there is a slate-blue or metallic cast like argyria. The brown pigmentation represents increased amounts of melanin in the epidermis and the metallic color is due to hemosiderin in the dermis. Melanin and iron can be seen microscopically with differential staining. Both iron and copper are deposited in the skin as part of the metabolic disturbance. Because these metals bind sulfhydryl groups, thus releasing the inhibition of tyrosinase, melanin formation is increased. In addition, hyperpigmentation may result from adrenal insufficiency or cirrhosis of the liver occurring as a complication of hemochromatosis.

It is often difficult to obtain good color matches in skin grafting. Sometimes the graft is lighter than the surrounding skin; in other patients the graft may be darker even though similar donor and recipient sites were used. (Fig. 11A and 11B.) While the basis for these changes is not known, it would be of interest to determine the influence of melanocyte population density on the resulting color of the graft.

In polyostotic fibrous dysplasia the following triad of abnormalities may occur: (1) disseminated but not generalized bone lesions, usually unilateral and segmental in distribution, (2) endocrine dysfunction associated with precocious puberty in females,[4] and (3) cutaneous pigmentation consisting of macules which vary greatly in size, tend to be of segmental distribution and are more extensive on the side of the bone lesions. They may be café au lait spots. It was stated that these lesions more so than those in neurofibromatosis were of irregular contour like the rugged "coast of Maine" whereas the café au lait spots of neurofibromatosis were described as being smooth-bordered like the "coast of California."[86] However, in a comprehensive study of neurofibromatosis which appeared recently it was shown that the configuration of the café au lait spots is variable and of no diagnostic significance.[53] Buccal pigmentation may occur. The etiology of the hyperpigmentation is not known but it may be neurogenic.

Intestinal polyposis consists of polyps distributed throughout the gastrointestinal tract, particularly the jejunum, associated with pigmentation of the buccal mucosa, lips, face and fingers. Pigmentation is more marked on the lower lip than the upper and more marked on the interior surface than the exterior. The pigmented lesions are macules, 0.2 mm. to 5 mm. in diameter, ranging in color from light brown to



11A 11B

FIG. 11. A, hyperpigmented skin graft. B, hypopigmented skin graft.

ep blue-black. Clinically, they appear to be nction nevi or lentigines and are observed first childhood. The syndrome is inherited as a nple Mendelian dominant.[87]

MELANOMAS

The term melanoma indicates a malignant owth so the prefix 'malignant' need not be pended. Because melanocytes are derived m neural rather than epidermal or meso-rmal structures the names melanocarcinoma d melanosarcoma are inaccurate. Melanomas ve been found in most animals; they are latively common in old gray horses. In some h, gray horses and other animals melanomas e transmitted genetically. This is not the usual se in human beings; only once were melanomas the skin reported in more than one member of family, although there have been four reports melanomas of the eye in families.[65]

Approximately 90 per cent of melanomas arise persons over thirty years of age.[66,67] They cur with equal frequency in both sexes. The cidence of melanomas is two to four times as eat in white persons as in Negroes. There is a neral feeling that the incidence is greater and e course more rapid in persons of light com-exion who do not tan but burn or freckle.

Melanomas have been reported in albinos ly twice.[68,69] In one case the tumor arose from pre-existing non-pigmented nevus and the es of metastases remained non-pigmented. the other a pigmented tumor arose from the e.

Melanomas are rare in children. However, a tal outcome may occur at any age.[70] Two ses were reported in which primary mela-mas originated prenatally and subsequently caused death of the infants.[71,72] Two other infants died of metastases from melanomas in the mother.[73,74]

In adults the melanoma is the commonest malignancy of the eye and metastatic melanoma is the commonest tumor of the heart. Pregnancy increases the growth rate of melanomas.[75]

Subungual melanomas occur with equal frequency on the hands and feet. However, they are more common on the thumb than on all other fingers combined and more common on the great toe than on all other toes combined. Nearly seventy cases of melanomas originating in the meninges have been described.[76]

Approximately 50 per cent of melanomas arise from pre-existing nevi.[66,77,78] In almost all instances it is the junctional and compound nevi which give rise to melanomas. Melanomas seem to come from nevi which have been subjected to pressure, trauma or sunlight. For example, melanomas have been known to develop in nevi following a hypodermic needle prick, chicken peck, vaccination and ice-pick stab.[79–82] Nevi of the face, genitalia, feet and nail bed are most likely to undergo trauma. In many cases a lesion is malignant prior to trauma or cauterization. Since more than 10 per cent of the population have some type of nevus on the palms and soles, it is not practical to excise all these lesions. The distribution of melanomas corresponds to the sites at which the nevi are most abundant, are subjected to trauma or are exposed to sunlight. In the distribution of melanomas[83] the percentage is as follows: trunk, 24; head and neck, 22; leg, 18; sole, 9; arm, 9; eye, 5; genitalia, 3; subungual, 3; oronasal and esophageal, 2; anorectal, 2; palm, 1, and primary site unknown, 2 per cent.

Melanomas are responsible for approximately 1 per cent of deaths caused by malignant tumors. Twenty-five per cent of deaths due to melanoma occur within the first year of the disease, 62 per cent between one and five years and 13 per cent after the tumor has been present more than five years. Approximately 14 per cent of patients with metastases to the regional lymph nodes survive five years. It is claimed that 40 per cent of patients without metastases to regional nodes survive five years. However, it is possible that not all primary lesions in this latter group were melanomas. Women tend to survive longer than men. Eye melanomas offer a better prognosis than those from other sites.[66,67,83]

Although the diagnosis of melanoma can be made clinically in certain obvious cases, microscopic examination is essential. Clinically, melanomas can simulate pyogenic granulomas, angiomas, seborrheic keratoses, nevi, basal or squamous cell carcinomas, Kaposi's sarcoma, foreign bodies, and the like. Some melanomas can be diagnosed microscopically with ease but in many cases the diagnosis cannot be made readily. Microscopically, a melanoma may show (1) an inflammatory infiltrate at the periphery of the lesion, (2) more than an occasional mitotic figure, (3) giant cells with a single bizarre nucleus, (4) marked pleomorphism, (5) general enlargement of all tumor cells and (6) tumor cells and mitoses in the epidermis.[84] Pigment may not be present.

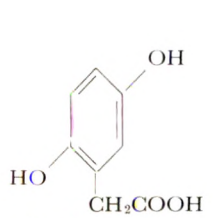
Melanoma patients often seem to have multiple primary tumors. That is, biopsy of a clinically benign nevus, distant from the primary melanoma, often shows malignant changes microscopically. Perhaps systemic changes occur in these patients so that the melanocytes appear more malignant.

The question has been raised as to whether or not it is possible to distinguish between melanomas and non-malignant pigmented lesions on the basis of the quantity or activity of tyrosinase present.[85] When large pieces of fresh melanoma tissue are incubated in solutions of tyrosine or dopa under the proper conditions, melanin is formed and the tissue turns dark. This experiment has not been done with melanomas from an albino. If tumors other than melanomas are incubated with dopa, pigment may form because dopa is oxidized readily. However, the reaction is much more specific with tyrosine.

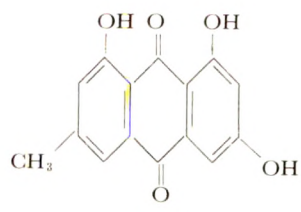
When the tyrosine and dopa reactions are carried out on thin sections of fresh melanoma tissue, microscopic examination shows that the substrates are converted to melanin. Here again the tyrosine reaction is more specific but less sensitive than that with dopa. Whether or not the total or active tyrosinase per melanocyte or melanin granule is greater in melanomas than in normal melanocytes remains to be proved.

NON-MELANOCYTE MELANIN

In this paper primary attention has been given to melanin formed from tyrosine when the principal reactions take place in the melanocyte. In considering natural pigments in microorganisms, plants and animals, it is necessary to define melanin in a broad sense as a dark pigment formed by the oxidation and polymerization of a polyhydroxy (or polyamino) aromatic compound with hydroxy or amino groups in the ortho or para positions. With this broad definition, one can say that the pigment in patients with ochronosis, phenol poisoning and melanosis coli is melanin. Ochronosis occurs in patients with alkaptonuria who have a genetic lack of homogentisase. They are unable to split the ring of homogentisic acid, which is a normal oxidation production of tyrosine in the liver, to form fumarylacetoacetic acid.[16] Homogentisic acid, a paradihydroxyphenyl compound, accumulates and is oxidized in tissues such as cartilage to a dark pigment. The oxidation probably occurs as a non-specific reaction in the presence of oxidizing systems such as cytochrome C-cytochrome oxidase.



Homogentisic Acid



Emodin

Exposure to phenol and related compounds can result in increased pigmentation of the skin. It is likely that polyhydroxy phenol derivatives are formed from phenol and oxidized to a pigment.

Melanosis of the large bowel occurs in persons who have used cascara as a laxative for long periods of time. Cascara contains emodin, an anthraquinone, which is probably oxidized in the presence of microorganisms in the colon to a black pigment that becomes attached to proteins of the mucosal wall.

SUMMARY

The mechanisms of normal and abnormal melanin pigmentation are discussed, with special reference to the cytology of melanocytes, enzymic factors concerned with melanin formation and hormonal and neurogenic control of melanocyte activity. An attempt is made to integrate these factors in order to explain clinical variations in pigmentation.

Acknowledgment: The author is grateful for those photographs furnished by Dr. A. G. Bearn, Dr. H. O. Curth and Dr. V. Lindgren.

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Seminar on Carbohydrate Metabolism

Evolution of Modified Insulins in the Treatment of Diabetes Mellitus, with Special Emphasis on Insulin-Zinc Suspensions*

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MULTIPLE modifications of insulin, particularly the protamine zinc, NPH and globin preparations, have been available for several years. Recently, the number of modified insulins has been further augmented by the introduction of the insulin-zinc suspensions or lente insulins. The physician must think of the effects of each modification and of certain combinations of them, not only in respect to the physiologic activity of insulin in general but also in terms of the three special dimensions of action, namely, promptness, intensity and duration. With so many variables to be considered the insulin situation is inherently confusing and a brief review, with emphasis on the new insulin-zinc preparations, seems to be in order.

The insulin-zinc suspensions have become available at a time when there is no pressing need in this country for such modifications. Physicians already have at their disposal preparations which, either singly or in combinations, have timing characteristics which fit reasonably well the needs of virtually all diabetic patients under almost all circumstances. Now, however, there is a plethora of modified insulins, with considerable duplication of the effects of certain preparations. As Colwell[1] has pointed out, had the lente modifications been discovered some twenty years ago it seems doubtful that any of the protein depot insulins ever would have emerged at all, for the newer preparations promise to provide all the gradations of timing of action that previously were offered by protamine zinc, NPH and globin insulin, alone or in mixtures with soluble insulin.

Nevertheless, the lente insulins are undoubtedly with us to stay and probably offer certain advantages over their predecessors. In this article an effort will be made to review the background for the development of these modified insulins, to comment briefly on their clinical use, and to indulge in some speculations regarding the future of modified insulins.

SOME GENERAL OBSERVATIONS

It is not always recognized that there is a great variability in response to treatment among diabetic patients who require insulin. The reasons for this variability are not well understood. Perhaps variations in response indicate that there are different forms of diabetes, different etiologic factors, or possibly only differences in the intensity of the disease. In any event, it is important to be aware of the inherent ease or difficulty of therapy in different patients before one chooses a program of insulin administration for any given patient, and particularly before one passes judgment on the scope of usefulness of any modification of insulin which is to be employed in a large group of patients. Hallas-Møller and his associates[2,3] have recently presented extensive data on the behavior of the blood sugar in response to various insulins in sixty-five patients with severe diabetes. Among other things, their data re-emphasize the varia-

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tions in response of different patients to the same insulin.

It should also be recognized that blood sugar curves, even if obtained under the most carefully controlled conditions, are not specific indexes of the action of the insulin under study but rather are an expression of the interaction of a number of factors. These include, in addition to the preparation of insulin employed, the patient as a whole, the type of diabetes which affects him, his diet, his physical activity or lack of it, his emotional state, the technic and site of injection of insulin, and perhaps other factors. In the brittle or unstable diabetic patient there may be variable and unpredictable alterations in some of these factors from time to time. Clinical experience has shown that no preparation of insulin can be expected to overcome all sources of variation in the behavior of this type of diabetes. It has often been stated that the ultimate goal in the development of insulin is an "automatic" insulin, that is, a preparation the rate of absorption of which from the site of subcutaneous injection will be determined by the level of the blood sugar or some other index of metabolic need. Unfortunately, there is no immediate prospect for the development of an insulin with such a high order of intellect and the physician must therefore make the best possible use of insulins which are currently available.

Any discussion of the niceties of action of insulin in clinical practice must be preceded by consideration of the question, Is control of diabetes important? This is not the place for a detailed discussion of this vital and controversial question. However, there is a growing impression, and some moderately convincing evidence, that poor control of sugar in the blood and urine may be a factor in the development of the degenerative complications of diabetes and, conversely, that good control of sugar in the blood and urine tends to prevent or delay the development of such complications. In any event, efforts to control diabetes seem to be advisable until such time as control has been conclusively shown to be unimportant. The discussion which follows is therefore predicated on the assumption that, in the present state of knowledge, control of diabetes is desirable.

EVOLUTION OF THE MODIFIED INSULINS

If one concedes that control of diabetes is a worth while objective, then the timing characteristics of the insulin or combination of insulins employed in each case assume considerable importance. Obviously, precise control cannot be achieved with a reasonably simple program of insulin administration unless the timing of the action of the insulin is well fitted to the needs of the individual patient.

The shortcomings inherent in the quick, intense and brief action of soluble insulin (regular and crystalline) and in the slow, weak and prolonged action of protamine zinc insulin are now generally appreciated. Intermediate-acting modifications were developed in response to a need for preparations the timing characteristics of which would be more consonant with the requirements of the majority of diabetic patients. The first such modifications which found wide use were mixtures of protamine zinc insulin and soluble insulin. The action and clinical use of such mixtures were thoroughly studied and described by Colwell and his collaborators.[4–6] Mixtures, when prepared extemporaneously in the syringe, provided necessary flexibility of quick and slow action for varying needs. They were used extensively in our own practice[7,8] and that of many others but obstacles were presented by their inconvenience and the difficulties which some patients experienced in mastering their use.

The combining of quick and prolonged actions in one insulin with intermediate timing characteristics was a step in the right direction. The first such insulin available for general use in this country was globin insulin.[9] Although controlled studies[10,11] showed its timing characteristics to be essentially similar to those of 2:1 mixtures of soluble insulin and protamine zinc insulin (the most commonly used proportions) and to those of NPH insulin, some observers gained the clinical impression that the action of a single morning dose of globin insulin was a little too short to maintain control of some cases of severe diabetes throughout the succeeding twenty-four hours.

NPH or isophane insulin[12] accurately reproduced the action of 2:1 mixtures[11,13–17] and promptly gained wide acceptance in this country. In our own practice, and that of many others, the majority of patients who had been under treatment with mixtures were changed to NPH insulin when the opportunity presented itself. Generally speaking, the results were good. When necessary, the timing characteristics of NPH insulin could be altered in the direction of greater speed by the admixture of small amounts of soluble insulin. For the few patients who

required a less intense insulin action during the day than was provided by NPH insulin, either the admixture of protamine zinc insulin with NPH insulin (which was rarely done) or the use of mixtures of soluble and protamine zinc insulin in a ratio of less than 2:1 was possible.

Thus insulins and combinations of insulins that fitted the needs of virtually all diabetic patients were at hand. Soluble insulin continued to be available for use in emergencies. NPH and globin insulin were well adapted to the day-by-day needs of most diabetic patients. The action of either could be accelerated, when necessary, by the addition of soluble insulin. Mixtures of protamine zinc insulin and soluble insulin could be adjusted to provide any other desired timing of insulin action. Insulin allergy was an infrequent problem. One could reasonably ask the question, What real need is there for additional insulin modifications?

It is apparent that if one thinks only in terms of the timing characteristics of insulin action, there was no pressing necessity for the introduction of the lente modifications. However, as will be brought out, these new modifications may provide slight advantages over other preparations, namely (1) a greater degree of "purity" owing to the absence of such modifying agents as protamine and globin, (2) a possibly diminished incidence of insulin allergy and (3) a possible ultimate reduction in the number of insulins from which the harried physician must choose.

The chemical characteristics of the currently available insulin modifications are shown in Table 1. It will be noted that the essential constituents of all the modifications prior to the lente insulins were insulin, zinc, a buffer (except in the case of globin insulin) and a modifying agent in the form of either protamine or globin.

THE LENTE INSULINS

Probably the most unusual aspect of Hallas-Møller's[18,19] discovery of the insulin-zinc modifications was his astute observation that the simple omission of phosphate or citrate buffer, and the employment of acetate buffer instead, enabled relatively small amounts of added zinc ion to exert a depressing effect on the solubility of insulin and hence on the rate of absorption of the hormone from sites of injection. Since the affinity of zinc for phosphate and citrate is greater than for insulin, the presence of these buffering agents effectively prevents or impedes the combination of zinc and insulin. Scott and Fisher[20] had shown in 1935 that the addition of rather large amounts of zinc to solutions of insulin containing phosphate buffer caused a pronounced prolongation of the action of the hormone when injected subcutaneously in rabbits. Rabinowitch and associates[21] had demonstrated the prolonged action of such preparations in diabetic patients. However, the amounts of zinc which it was necessary to add were large (400 to 1,000 mg. per 1,000 units) and the preparations were not considered suitable for clinical application.

TABLE 1
CONTENT OF ZINC, BUFFER AND MODIFYING PROTEIN OF VARIOUS INSULINS

Insulin	Description	Zinc Content (mg. per 1,000 units)	Buffer	Modifying Protein
Short Action				
Amorphous	Solution	0–0.4	None	None
Crystalline	Solution	0.16–0.4	None	None
Semilente	Suspension	2–2.5	Sodium acetate	None
Intermediate Action				
NPH	Suspension	0.16–0.4	Sodium phosphate	Protamine
Globin	Solution	2.5 –3.5	None	Globin
Lente	Suspension	2 –2.5	Sodium acetate	None
Prolonged Action				
Protamine zinc	Suspension	2 –2.5	Sodium phosphate	Protamine
Ultralente	Suspension	2 –2.5	Sodium acetate	None

Hallas-Møller's work elucidated a new concept of the chemical relation of zinc and insulin. It conclusively demonstrated that the solubility of insulin can be lowered and its action prolonged simply by the addition of zinc under appropriate chemical conditions, without recourse to protamine or comparable basic substances. In the lente insulins the concentration of zinc, although remarkably low, is nevertheless approximately ten times the amount necessary for the formation of soluble zinc-insulin crystals. (Table 1.) By careful adjustment of the pH a number of crystalline and amorphous preparations can be produced. The crystalline compounds are much more insoluble than the amorphous, and therefore much longer-acting.

Lente Preparations. Of the many different types of insulin-zinc suspensions which can be prepared under different chemical conditions, three have now had clinical trial. The name given to each utilizes the basic term "lente" to indicate slow action.

1. *Semilente*, which is a suspension of zinc-insulin crystals in an amorphous state. Its action is intermediate between the actions of soluble insulin and NPH insulin in terms of promptness, intensity and duration.

2. *Ultralente*, which is a suspension of zinc-insulin crystals of a size of 10 to 20 μ. Its action is slow, prolonged and of low intensity, similar to that of protamine zinc insulin.

3. *Lente*, which is a mixture of the foregoing two preparations in proportions of 30 per cent semilente and 70 per cent ultralente. As already indicated, the timing of its action is almost precisely the same as that of NPH insulin.

Inasmuch as the lente modification or mixture is the only one currently available on the market in this country, and the one which has had the most extensive clinical trial here and elsewhere,[2,3,22–65] most of the comments which follow will be limited to this preparation. In addition, some predictions of the ultimate place of semilente and ultralente modifications, which no doubt soon will be available, will be made.

Experience to date indicates that lente insulin and NPH insulin can usually be used interchangeably. In an occasional case the action of lente insulin has seemed a little less intense and more prolonged than that of NPH insulin. Generally speaking, however, it is our current impression that one must look elsewhere than timing characteristics for advantages of lente insulin over other preparations with intermediate action.

Is the "Purity" of Lente Insulin an Important Advantage? Lente insulin, containing as it does no modifying protein, fits in with a developing trend toward purity in drugs and hormones for clinical use. That such a trend actually exists is attested by the gradual replacement in clinical medicine of such agents as digitalis leaf by pure cardiac glucosides, relatively crude adrenocortical extracts by synthetic steroid hormones in crystalline form, plant antispasmodics such as belladonna by a variety of synthetic antispasmodics, and many similar examples. At present, it is difficult to assess the importance of the purity of lente insulin. It has been speculated that prolonged administration of protamine in the form of protamine zinc insulin or NPH insulin might be a factor in the development of the degenerative complications of diabetes but there is actually little or no basis for this supposition. With the possible exception of the incidence of insulin allergy, which is low with any insulin, there are no good yardsticks for the appraisal of the importance of the purity of lente insulin.

Insulin Allergy. The literature on lente insulin to date suggests that its use is probably attended by a lower incidence of local and general sensitivity reactions than in the case of insulins containing modifying proteins. This is what would be anticipated, for protamine and globin are undoubtedly responsible for some of the sensitivity reactions to insulins containing these substances. However, it seems likely that any insulin made from animal pancreas, no matter how pure it might be, will be antigenic in a small number of diabetic patients. Hallas-Møller and associates[18] and others (Murray and Wilson,[32] Nabarro and Stowers,[33] Jersild and associates,[22] Lawrence and Oakley,[24] and Oakley[31]) have apparently not observed any instances of insulin allergy among patients treated with lente insulin. Their reports were based mainly on experience with the Danish preparation. Subsequent reports by some of these investigators and others (Armstrong and Lloyd[48] Greenhouse,[60] Holcomb and associates,[45] Peck and associates,[47] Gurling and associates,[65] Stowers and Nabarro[59]) have indicated that the British and American lente insulins are capable of inducing sensitivity reactions. It appears that an insufficient number of cases in which lente insulin from various commercial sources has been used and which have been carefully observed with respect to the development of insulin allergy have yet been reported to provide an estimate of the incidence of sensitivity reactions.

Modification of the Timing Characteristics of Lente Insulin. Because of its intermediate timing characteristics, lente insulin, like NPH insulin, is undoubtedly a suitable preparation for a high proportion of all diabetic patients who require treatment with insulin. There are, however, a few patients for whom its action is either too slow and prolonged or too rapid and short. For these patients the action of lente insulin must be either accelerated and shortened or retarded and prolonged.

Limited experience to date indicates that acceleration of action can be achieved by mixing

small amounts of soluble insulin with lente insulin. Apparently the independent actions of the two insulins are preserved in the mixture, provided that the amount of added soluble insulin is small. Colwell[1] has cautioned that the addition of large amounts of soluble insulin to lente insulin may alter the size and form of the insulin-zinc crystals in an unpredictable manner, and therefore the rate of release of the hormone from sites of injection. In other words, not enough is yet known about the action of mixtures of soluble and lente insulin in varying proportions to warrant final statements regarding their clinical application.

TABLE II
USE OF LENTE INSULINS AT HVIDØRE HOSPITAL, COPENHAGEN, DENMARK (REPORT OF JULY, 1954)*

	Patients	Per cent
Adjusted on the lente insulins..... (Average daily dose, 40 units)	1,030	100†
Dismissed with one injection daily..	990	96†
Dismissed with two injections daily.	40	4†
Types of Insulin Employed in the Group Receiving One Injection		
Semilente alone.....	0	0‡
Lente-semilente mixtures.....	93	9‡
Lente alone.....	790	80‡
Lente-ultralente mixtures.....	100	10‡
Ultralente alone.....	7	1‡

* Data from Hallas-Møller.[66]
† Per cent of 1,030.
‡ Per cent of 990.

For the future, a more promising method for tailoring the timing of insulin action to the needs of individual patients may be the preparation of mixtures containing semilente and ultralente insulins in varying proportions. The two insulins can be mixed in any desired proportion without altering the chemical or physical properties of either. Ultralente and semilente in a 70:30 proportion (lente insulin) fit the needs of a high proportion of all patients who require insulin. It should be possible to regulate patients who require a more rapid insulin action during the day on mixtures containing a higher proportion of semilente insulin, while the few who require a slower action can be regulated on mixtures containing a higher proportion of ultralente insulin. Hallas-Møller has recently reported on the frequency with which lente-semilente and lente-ultralente mixtures have been employed in the treatment of a large number of diabetic patients in Denmark. (Table II.)

The desired proportion of semilente and ultralente insulin in a mixture can be obtained either (1) by mixing semilente and ultralente insulin or (2) by mixing lente with either semilente or ultralente insulin. Careful education of the medical profession, to say nothing of diabetic patients, will be necessary if confusion is to be avoided in the preparation of such mixtures.

OBSERVATIONS ON 100 CONSECUTIVE PATIENTS TREATED WITH LENTE INSULIN

A review of the records of 100 consecutive ambulatory diabetic patients who have been treated with lente insulin at the Mayo Clinic since January 1, 1955, tends to confirm many of the foregoing observations.* Most of the 100 patients had not previously been under our care and came to us on a variety of different programs of insulin administration. The principal reasons for the decision to treat them with lente insulin were as follows: (1) Inadequate control of daytime glycosuria and occurrence of nocturnal hypoglycemic reactions during treatment with protamine zinc insulin alone or with mixtures of protamine zinc insulin and soluble insulin which contained too high a proportion of protamine zinc insulin (twenty-four patients). (2) Greater convenience of one daily dose of lente insulin as compared with mixed doses of protamine zinc insulin and soluble insulin (five patients). (3) Inadequacy or inconvenience of multiple doses of soluble insulin (four patients). (4) Daytime reactions and inadequate overnight control during treatment with globin insulin in single morning doses (four patients). (5) Local allergic reactions to NPH insulin, protamine zinc insulin or soluble insulin (six patients). (6) Patients arbitrarily changed from NPH insulin or protamine zinc insulin to lente insulin for no clear-cut reason (sixteen patients).

* Supplies of lente insulin for preliminary studies were kindly provided by the Lilly Research Laboratories, Indianapolis, Ind.

(7) Patients not previously treated with insulin and arbitrarily chosen for treatment with lente insulin (twenty-six patients). (8) Patients already under treatment with lente insulin when first seen (fifteen patients).

It will be noted that the reasons for the employment of lente insulin in preference to other intermediate-acting insulins were usually not of a compelling nature. The principal gains from the change to lente insulin, if any occurred, were increased convenience and improvement of control. In most instances one or both of these advantages could as well have been achieved by the use of one of the other intermediate-acting insulins. For example, patients whose previous control with protamine zinc insulin alone had been inadequate could have been treated as well with one of the other intermediate-acting preparations as with lente insulin. There was no discernible advantage in changing from NPH to lente insulin. In a few instances, lente insulin seemed to provide better control than globin insulin by virtue of a less intense and more prolonged action.

Observations in Six Cases of Insulin Allergy. As already indicated, six of the patients who were treated with lente insulin had experienced local allergic reactions to the preparation which they had previously employed. None of these derived complete relief by the substitution of lente insulin. Two felt that their local reactions were as intense as before, while in the remaining four patients slight to marked diminution in the intensity of the local reaction was reported. Among the latter four patients was one with insulin atrophy. Subsequent observation of this patient, however, indicated that lente insulin was producing mild but definite atrophy as well as local sensitivity reactions.

Mixtures of Lente and Soluble Insulin. Such mixtures were employed in seven cases. In each of these the ratio of units of lente to units of soluble insulin was high, the lowest ratio being 7:3. As far as could be determined, the use of relatively small amounts of soluble insulin produced a satisfactory acceleration of insulin action without noticeably altering the depot characteristics of the lente insulin. The possibility exists, however, that employment of larger amounts of soluble insulin in such mixtures, with consequent alteration of pH, might change the character of the crystals of lente insulin and therefore their depot effect.

The Use of Two Doses of Lente Insulin. It has long been our belief that improved control can be achieved in a small group of patients with unstable diabetes by the use of two daily doses of intermediate-acting insulin. It has been our practice to administer the two doses before breakfast and before supper; others have administered the evening dose at bedtime. We know of no data which demonstrate which of these methods is to be preferred.

In the present series of 100 patients five were treated with two daily doses of lente insulin. All five had unstable diabetes which had not been well controlled on any program involving a single morning dose of insulin. The morning doses were from two to six times as large as the evening doses. In one instance the morning dose was a mixture of lente and soluble insulins. In the other five instances lente insulin alone was employed.

The advantages of the use of lente and other intermediate-acting insulins in this manner in some cases of unstable or brittle diabetes seem to outweigh the disadvantage of two daily injections of insulin. In our opinion, it is a method of treatment which deserves more extensive application to severe diabetes than it now has.

THE FUTURE PLACE OF INSULIN-ZINC SUSPENSIONS

For the many physicians who treat only a few diabetic patients and who find it difficult to focus their attention on the finer subdivisions of the insulin field, the addition of three new modifications of insulin to those already available is more likely to be confusing than helpful. From this point of view, the introduction of semilente, lente and ultralente insulin is to be viewed with some misgivings, especially since their advantages over existing preparations are not great and, indeed, in the individual case will usually be difficult to recognize or demonstrate.

On the other hand, with the passage of time it is not unlikely that the new preparations, by substituting for and replacing existing preparations, may help to bring about a reduction in the number of insulins from which the physician must choose. Insulin manufacturers might well do what they can to hasten the replacement of some of the existing preparations with the lente insulins. Certainly the new insulins will make it possible for patient and physician to obtain any desired timing of insulin action either by using lente insulin alone or by using semilente and ultralente insulin in proper proportions. As Colwell[1] has pointed out, it remains to be determined whether predictability of action and consistency of response to these insulins will be

greater than in the case of the protein-precipitated insulins. If the insulin-zinc suspensions are not inferior in this respect, and there is no reason to anticipate that they will be inferior, there is no compelling reason why they should not eventually take the place now held by the protein-precipitated insulins in the therapy of diabetes. They will not, of course, replace soluble insulin for the treatment of emergencies such as diabetic acidosis.

SUMMARY

For some time the physician who is concerned with the treatment of diabetes has had at his disposal good preparations of insulin with timing characteristics which are suitable for almost all diabetic patients under almost all circumstances. In the past fifteen years there has been increasing appreciation of the fact that insulins with timing characteristics intermediate between those of soluble insulin and protamine zinc insulin provide excellent replacement therapy for the great majority of patients who require insulin. These preparations include NPH insulin, globin insulin and appropriate mixtures of soluble insulin and protamine zinc insulin.

While one's first reaction to the introduction of the new insulin-zinc suspensions is likely to emphasize the confusion resulting from the availability of additional insulins, it is not unlikely that the new preparations, by virtue of their freedom from modifying proteins and their probable adaptability to the needs of almost all diabetic patients, may eventually substitute for and replace all of the existing preparations except regular insulin and crystalline insulin. Such a course of events, while it might take a long time, could be accelerated by the insulin manufacturers and would simplify a confusing situation.

Observations on 100 consecutive patients with diabetes mellitus treated with lente insulin are presented.

With the introduction of the insulin-zinc suspensions, it is difficult to conceive of any further important practical developments in the field of injectable modified insulins, short of an "automatic" insulin whose rate of release from the site of injection would be determined by the level of the blood sugar. We know of no prospect for the development of such an insulin in the foreseeable future. At present the most fruitful field for improvement of the treatment of diabetes is the clinical use of currently available insulins to the best possible advantage of the patient.

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The Problem of Degenerative Vascular Disease in Diabetes*

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VASCULAR disease is generally recognized as the most important problem in clinical diabetes today. Seventy per cent of diabetic deaths in Joslin's clinical cases[2] and 47 per cent in Bell's[3] postmortem series were caused by arteriosclerosis. By comparison, 30 per cent of deaths in the general population are ascribed to this cause.[3] What is more striking, indeed appalling, is the high incidence of vascular degeneration in young patients with long-standing diabetes. White and Waskow,[4] for example, found that of 200 such patients whose diabetes had begun in childhood and who had survived for twenty years or more, 92 per cent were so affected. It is apparent that senescence plays a far lesser role than does diabetes itself, or some factor associated with it, in the vascular disorders of this disease.

THE LESIONS AND THEIR CLINICAL EFFECTS

The clinically important vessels involved are for the most part the medium-sized arteries of the myocardium, lower limbs and brain; the arterioles, especially of the kidney; and the capillaries of the renal glomeruli and retina.

Atherosclerosis and Arteriolosclerosis. The characteristic lesion of the medium-sized arteries in diabetes is atherosclerosis.[5] This term is used to describe focal thickenings of the intima which contain greater or lesser amounts of lipids and, in the later stages, calcium. It is these lesions that are responsible for most of the occlusive vascular phenomena which constitute the largest single cause of death in the diabetic population.

Arteriolosclerosis of the kidney, affecting particularly the afferent and efferent vessels of the glomeruli, is marked by hyalinization of the arteriolar wall. Although often observed in other conditions, such as chronic glomerulonephritis and essential hypertension, it occurs with greater frequency and with much greater intensity, according to Bell,[6] in long-standing diabetes, particularly in younger patients with hypertension.

Intercapillary Glomerulosclerosis. Notwithstanding the importance of disease of the arterial system, which after all occurs commonly enough in non-diabetic persons, disease of the capillaries has received increasing attention because certain forms of it are almost uniquely an accompaniment of the diabetic state.

Characteristic alterations of the renal glomeruli were described in association with diabetes in 1936 by Kimmelstiel and Wilson,[7] who called the condition "intercapillary glomerulosclerosis." Their findings were quickly and widely confirmed but there has been disagreement as to whether the lesions arise between the glomerular capillaries, as the original term implies, or in the basement membrane of the capillary itself. At any rate there occurs, probably as the earliest change, an axial thickening in the supporting structure of the glomerular tuft. This has been referred to as the diffuse form of the disease,[6] and it may progress in extent and severity without the development of nodules. In many cases, however, the process goes on, by steps as yet unknown,[8] to the formation of spheroid nodules resembling hyaline. These stain pink with hematoxylin and eosin and readily accept the periodic acid-Schiff reagent, a property which suggests that they contain a carbohydrate, probably a mucopolysaccharide.[9] That they are not composed of the usual hyaline is indicated by the fact that they are more resistant than this material to digestion with trypsin.[10] Some of the nodules are laminated, and many of them contain lipids, according to Wilens,[11] who has proposed that the deposition of fatty substances may be primary in their pathogenesis. Dilatations of the capillaries,[12] which have been

* For a more detailed treatment of this subject, as well as a more comprehensive bibliography than this article provides, the reader is referred to the excellent review of LeCompte.[1]

likened by Friedenwald[13] to the microaneurysms of the retina, have been described.

The diffuse type of glomerular involvement, while seen far more commonly in diabetes than in other conditions, does not seem to be specific for that disease for similar lesions are found in some cases of glomerulonephritis[6,14] and in aged individuals.[15] The nodular type, however, particularly when severe, is for all practical purposes confined to the kidneys of diabetic patients.[10]

The frequency with which intercapillary glomerulosclerosis is found among diabetics at necropsy has been variously reported to be from 20[16] to 64[17] per cent. This variation is doubtless explained in large part by differences in the stringency of the histologic criteria adopted for diagnosis.

The clinical manifestations of intercapillary glomerulosclerosis, when fully developed, consist of albuminuria, edema and hypertension. Retinopathy, which is basically "diabetic" in appearance and origin but may have "hypertensive" elements, is the rule. Doubly refractive fat, both in the free form and in casts, is usually found in the urine.[18] Anemia is common, hypoproteinemia less so, and azotemia appears with the advent of renal insufficiency.

Considering the difficulties of fixing the time at which the renal disease actually begins, it is safe to state that its course is relatively slow. Henderson and his collaborators,[14] in a review of postmortem records, found that patients survived for months to years after the syndrome had been recognized, whereas the terminal illness of patients with glomerulonephritis was of one to four months' duration. The chronology of events in thirteen fatal cases of "diabetic nephropathy" (*vide infra*) has been summarized by Wilson, Root and Marble as follows:[19] In terms of average patient age, diabetes began at 14.2 years, "nephropathy" at 22.0 years and death occurred at 33.0 years. Among the sixty-one cases studied by Henderson[14] death was caused by cardiovascular disease in 52.5 per cent and by renal insufficiency in only one case. It is important to realize, however, that the average age of these patients at death was sixty years. In studies of younger patients based on clinical records, Root[20] found "nephritis" to be the cause of death in 30 per cent and heart disease in 32 per cent of 531 patients with onset of diabetes prior to thirty years of age and duration over fifteen years; and Joslin and Wilson[21] report death from "nephritis" in 52 per cent of 135 juvenile diabetic patients who died between 1944 and 1949. Thus renal disease seems to account for a higher proportion of deaths as age at death decreases. It should be noted that the term "nephritis," and later "nephropathy," (as used by the Joslin group) includes nephrosclerosis and pyelonephritis as well as intercapillary glomerulosclerosis. This renders impossible a strict comparison of their clinical data with those on only intercapillary glomerulosclerosis obtained at necropsy. Such terminology, however, seems unavoidable in view of the lack of complete correlation between clinical manifestations and the histologically proved presence of the Kimmelstiel-Wilson lesion. Biopsies of the kidney during life,[22,23] as well as postmortem studies,[1,24] reveal that not all patients with this lesion exhibit symptoms and not all patients with suggestive symptoms have the lesion.

Zubrod, Eversole and Dana[25] have made the interesting observation that diabetes tends to be, or become, mild and acidosis rare in individuals with proved intercapillary glomerulosclerosis. However, the fact that younger patients with long-term diabetes of great severity—a group but scantily represented in that study—have a high incidence of renal disease prevents acceptance of the authors' conclusions as being generally applicable. They have indeed been challenged by Runyan and co-workers[26] who reported in a similar study of necropsy and clinical records that patients with and without the specific glomerular lesion experienced much the same changes in their insulin requirements as the illness became terminal, approximately 26 per cent of both groups needing more, 38 per cent less and 34 per cent the same amount of insulin as compared with their earlier requirements. The latter authors also found that twenty-five episodes of acidosis had occurred in the fourteen patients with specific renal disease and eleven episodes in the nine patients without it. They have provided a probable explanation for the diminished insulin requirement in some cases by following the dietary histories of five living diabetic patients during the onset and progression of kidney disease and demonstrating a marked diminution of food intake. They suggest that failure to lose weight, cited by Zubrod as evidence against a decreased consumption of food, may be due to progressive edema.

Diabetic Retinopathy. Retinopathy comprises the other great class of capillary lesions in diabetes. Its earliest manifestation is the appear-

ance of tiny saccular dilatations arising from the venous side of the capillary bed in the inner nuclear layer of the retina. These microaneurysms, as they have been termed by Ballantyne and Lowenstein,[27] vary in size from 30 to 90 μ in diameter, hence many of them can be seen with the ophthalmoscope. Some of them are undoubtedly what used to be described as "punctate hemorrhages." While the latter certainly occur, and definite distinction is often difficult during life, the microaneurysms have borders that are sharply circumscribed instead of fuzzy and they may be seen in exactly the same place for months or years instead of undergoing absorption and scarring. The walls of some are thin and presumably rupture easily, while others have thick walls that appear to contain hyaline, an observation which suggested to Friedenwald[13] that these aneurysms and the glomerular nodules of the Kimmelstiel-Wilson lesion may be chemically, and even pathogenetically, related. In addition to the capillaries, the veins are frequently involved, being rather uniformly dilated in the early stages and later showing marked tortuosity and beading. Sclerosis of the arteries also occurs but is less specific and less striking than the venous lesions. Disease of both capillaries and veins leads to retinal hemorrhages which vary in size from minute to massive, sometimes invading the vitreous. Exudates, which appear "hard" and yellowish in contrast to those of hypertension and glomerulonephritis, are of common occurrence. Extensive growth of new vessels (retinitis proliferans) and scar formation may result in retinal detachment.

As in the case of intercapillary glomerulosclerosis, retinal microaneurysms are not entirely specific for diabetes but they are seen far more commonly in this condition than in others.[28] They have been reported in cases of severe hypertension, in patients with glaucoma and even in supposedly normal individuals,[29] and have been observed both experimentally and clinically in occlusion of the central vein of the retina.[30] What role, if any, venous stasis plays in the production of microaneurysms in diabetic persons is conjectural. Certainly they occur frequently in the absence of both arterial hypertension and retinal arteriosclerosis.[28]

Henderson and his colleagues[14] have called attention to the disturbing increase in the incidence of retinopathy among diabetic patients as revealed by a succession of studies at the Mayo Clinic. In 1921 the frequency was 8.3 per cent; in 1934, 17.7 per cent and in 1945, 30.6 per cent. These figures are for all age groups and, moreover, do not take account of the duration of diabetes which is a factor of the greatest importance in determining the development of retinopathy. It is surely, at least in part, the short duration of life, as well as of diabetes, that explains the low incidence of retinopathy in 1921, just as the much longer duration of both contributes to its high incidence in the present day.

The clinical symptoms of retinopathy vary from minimal disturbance of vision to complete blindness. The latter may occur within a few months of onset but usually the course of the disease is measured in years. The author has observed a number of patients with minimal retinopathy which has apparently remained stationary over periods up to five years. In the more severe cases secondary glaucoma is not uncommon

The close, although not universal, association between diabetic retinopathy and intercapillary glomerulosclerosis is well established. Henderson[14] reports retinopathy in 68.8 per cent of cases in which the presence of the nodular renal lesions was proved by necropsy but in only 22.8 per cent of cases in which the absence of such lesions was so proved. This means that nearly one-third of patients with unquestionable intercapillary glomerulosclerosis had no clinically recognizable retinopathy. It must be pointed out, however, that retinopathy, or lack of it, in these cases had been determined ophthalmoscopically during life. When Ashton,[12] and later Friedenwald,[31] examined both the kidneys and the retinas of diabetic patients microscopically, no Kimmelstiel-Wilson lesions were found in the absence of retinal microaneurysms. On the other hand, the existence of retinopathy does not guarantee the presence of the renal lesions, for when the kidneys of patients with microscopically demonstrated retinal aneurysms were examined intercapillary glomerulosclerosis was found in only 58 per cent by both Ashton and Friedenwald.

The involvement of the capillaries of the kidney and retina led Ashton to a search for similar lesions in other tissues.[29] The results were negative. While increased capillary fragility has been demonstrated by the tourniquet test in patients with diabetic retinopathy,[32] structural changes in the cutaneous capillaries have not been described. Ditzel[33,34] has reported

abnormal patterns in the conjunctival vessels of living diabetic patients and, in some cases, their blood relatives. This potentially important observation awaits confirmation.

CIRCUMSTANCES AFFECTING THE OCCURRENCE OF VASCULAR DISEASE IN DIABETES

Heredity. Primary among the many questions of which our problem is composed is that of whether susceptibility to vascular degeneration is a result of the diabetic state or whether it is hereditary or constitutional, being passed on to succeeding generations along with the tendency to diabetes itself. Conceivably, both might be true, one factor reinforcing the other. If diabetes alone were responsible, one would expect to find a correlation between the frequency and severity of vascular disease on the one hand and, on the other, the duration and magnitude of the metabolic disturbances. There is in fact some such correlation and its importance is very great but it appears to fail in a significant number of cases. Vascular lesions are seen in some patients with presumably early and certainly mild diabetes and are sometimes not demonstrable in patients whose diabetes is of long standing, severe and habitually under poor control. It is these facts that have raised the question of an hereditary influence. Actually, meaningful data on this subject are non-existent so that it must remain for the present a topic for speculation.

On the other hand, there is evidence in both man and animals that diabetes *per se*, accidentally or intentionally produced under conditions which nearly or completely exclude genetic influences, sometimes leads to vascular degeneration. Lawrence[35] cites cases of this kind in which diabetes was brought on by gross pancreatic disease, and Sprague[36] has described a patient in his early forties with a negative family history in whom diabetes appeared during the course of chronic relapsing pancreatitis, followed by neuritis after seven years, diabetic retinopathy after ten years and the manifestations of intercapillary glomerulosclerosis after eleven years of diabetic symptoms. Hudson[37] has reported retinal microaneurysms in a fifty year old diabetic patient with hemochromatosis of fifteen years' duration. The experimental induction of diabetes has been followed by sclerosis of the aorta[38] and coronary arteries[39] in dogs and by lesions resembling intercapillary glomerulosclerosis in dogs[40,41] and rats.[42,43] No claim has been made in any case that these lesions were identical with those found in man but the observations remain highly significant nevertheless.

Sex. Coronary disease among non-diabetic persons occurs several times more frequently in males than in females. Diabetes not only increases by two- or threefold the over-all incidence of this disorder but also eliminates the sex difference, so that the ratio of males to females experiencing myocardial infarction is approximately one to one.[44–47] The same is true for arteriosclerosis of the legs, which among non-diabetic persons is observed predominantly in males[48,49] but among diabetic persons, as evidenced by gangrene, occurs as often in women as in men.[47,50]

Although some authors have reported no sex difference in the frequency of "chronic renal disease"[47] and "nephritis"[51] among diabetic individuals at necropsy, the majority who have confined their studies to intercapillary glomerulosclerosis have found the percentage of occurrence from one and one-half to two times as great in women as in men.[6,15,46,52]

Diabetic retinopathy seems to afflict males and females with about equal frequency.[47,53] This is surprising in view of the close relationship between retinopathy and intercapillary glomerulosclerosis and the somewhat greater predilection of the latter for women.

The Time Factor: Age of Patients and Duration of Diabetes. It is difficult to discuss the effect of age on vascular disease without considering the effect of duration of diabetes, for the latter, at any given age, is a factor more potent than any other we know, and within different age groups has different degrees of influence.

Age alone, of course, plays its own role, particularly in arterial disease. It is evident from Table 1, compiled from the report of Root and his colleagues,[44] that the incidence of coronary occlusion at necropsy is nearly four times higher in diabetic patients between forty and sixty years of age than in those under forty. The corresponding figures for non-diabetic persons, however, show a tenfold increase in the older as compared with the younger ages, indicating that advanced age is of less importance in the presence of diabetes than in its absence. Conversely, for ages under forty diabetes increases the frequency of coronary occlusion tenfold while for those over forty the increase is only fourfold. Diabetes, then, has a more profound effect on coronary disease in younger persons than in

older ones. It seems to have a similar effect on peripheral arteriosclerosis in relation to age, for Bell[50] has shown that the incidence of gangrene is 156 times greater in diabetic than in non-diabetic persons between the ages of forty and fifty but only eighty-five times greater at ages fifty to sixty and fifty-three times greater at ages sixty to seventy.

TABLE I
INCIDENCE OF CORONARY OCCLUSION AT NECROPSY IN OLDER AND YOUNGER SUBJECTS WITH AND WITHOUT DIABETES*

	Per cent of Cases	
	Age under 40	Age 40 to 60
Diabetics	6	23
Non-diabetics	0.6	6

* From Root, Bland, Gordon and White.[44]

Concerning the vascular lesions that are more specific for diabetes, some studies indicate that intercapillary glomerulosclerosis occurs predominantly in the older ages.[14,52] No doubt this is true, for the older diabetics outnumber the younger, but there is a growing realization, as pointed out by Wilson, Root and Marble,[19] that the highest *incidence* of this form of renal disease is in patients in their thirties and forties with long-standing, poorly controlled diabetes. In Goodof's study[15] the frequency of the Kimmelstiel-Wilson lesion rose with age between ten and thirty years but then leveled off until age seventy when a rise occurred in both diabetics and non-diabetics. There was no appreciable difference in incidence between age thirty and ages sixty to seventy, suggesting that senescence does not increase susceptibility to this disorder. The same seems to be true for retinopathy.[28,47,54]

The dominant part played by long duration of diabetes has been thoroughly established.[3,4,6,15,20,28,51,55,56] The studies of White and Waskow[4] are most striking. Among 200 patients having onset before the age of fifteen and surviving for more than twenty years they found the following: "nephritis" in 50 per cent, calcified arteries in 75 per cent and retinal hemorrhages in 80 per cent. These consequences or concomitants of diabetes are uncommon in the early years of the disease but between the tenth and twentieth years of diabetes they assume alarming proportions. After twenty to twenty-five years increasing duration is less effective in augmenting the frequency of vascular lesions.[6,57]

The inter-relationship between duration of diabetes and age at death as they affect renal disease is brought out by Bell.[6] Table II, taken from his data, shows that for durations under ten years the incidence of intercapillary glomerulosclerosis increased from 3 per cent at ages twenty to forty to 20.3 per cent at ages sixty to ninety, whereas for durations over twenty years it decreased, oddly enough, from 100 per cent at ages twenty to forty to 41.4 per cent at ages sixty to ninety. Expressed in other terms, for short duration age increases frequency sevenfold, while for younger ages long duration increases frequency thirty-three fold. Bell's data[6] for renal arteriolosclerosis reveal similar trends, as do those of Root[20] for "nephritis." Here again it is evident that long-standing diabetes has its greatest effects in younger patients.

TABLE II
INCIDENCE OF INTERCAPILLARY GLOMERULOSCLEROSIS AT NECROPSY IN RELATION TO AGE AT DEATH AND DURATION OF DIABETES*

Age at Death (yr.)	Per cent of Cases		
	Duration Less than 10 yr.	Duration 10 to 20 yr.	Duration Over 20 yr.
20 to 40	3	51.4	100
40 to 60	19.8	41.7	65
60 to 90	20.3	36.5	41.4

* From Bell [6]

Finally, despite what has just been said, long life with diabetes does not necessarily spell disaster. Bell[3] found that twenty-eight of ninety-three persons who lived more than twenty years after onset did not have serious vascular disease at necropsy. And although 92 per cent of White's[4] long-term patients had lesions of one sort or another, many of these were not severe and 8 per cent of the patients had none.

Severity of Diabetes. The statement is often made that severity of diabetes has no relation to the occurrence of vascular lesions.[6,14,24,28] This statement needs examination. How is severity determined? Usually it is judged by insulin requirement because this is the only way in which it can be expressed in numerical terms.

No one would deny that the diabetic patient who requires no insulin has the disease in mild form. It is even reasonable to suppose that in patients who do take insulin there is some rough, over-all correlation between severity and insulin dosage. But instances in which this correlation fails are fairly numerous and very important. The true test of severity is the behavior of the disease when insulin is withheld. There are many patients, chiefly those with onset of diabetes at a young age, who require doses of less than 40 units daily but in whom acidosis develops promptly when treatment is stopped. On the other hand, many patients with high requirements, principally in the older age groups, tolerate the withdrawal of insulin for days or weeks with no more than moderate glycosuria. Surely the former group represents the more severe degree of diabetes, and it is in these younger patients with long-standing disease that the incidence,[19] and one might add the seriousness, of vascular disease is most marked. The use of insulin requirement as an index of severity, and the preponderance in postmortem material of older subjects with mild diabetes but a considerable incidence of vascular lesions, due in part to aging, have no doubt contributed to the impression of pathologists that such lesions are as frequent in mild as in severe diabetes. Illuminating and indispensable as the observations of the pathologist are, it might be questioned whether he is in a position to evaluate severity as well as the clinician.

Although severity is probably more important in predisposing to vascular disease than has been generally realized, it must be granted that a good many patients with indisputably mild diabetes suffer from degenerative changes while others whose disease is obviously severe escape.

Control of Diabetes. The argument over the role of control is much like that over the role of severity, except that it is more heated, and the same kinds of questions present themselves. What is control? Are there more than a handful of "juvenile" diabetics who *can* be well controlled at all times? Is good control in severe diabetes the equivalent of poor control in mild diabetes? Can the pathologist, or anyone else for that matter, tell from a retrospective search of clinical records what the *habitual* degree of control has been in a patient who has seen his physician on only one day of thirty, ninety or 365 over a period of twenty years? The lack of satisfactory answers to these questions should be borne in mind in reviewing reports which attempt to relate degree of control of glycosuria to the occurrence of degenerative disease.

Perhaps it is well to start by reminding ourselves that in the few instances in which arterial and glomerular lesions have been observed in experimental diabetes, glycosuria has been relatively or entirely uncontrolled.[38–43] Similar experiments in which good control had been carefully maintained have not been described.

The notion that all diabetics, if they live long enough, eventually fall prey to vascular disease received strong support from the findings of Dolger in 1947.[54] In a group of 200 patients examined at frequent intervals for periods up to twenty-five years, none escaped retinopathy "regardless of age of onset, severity of diabetes or type of treatment used." Fifty per cent had hypertension and clinical evidence of renal disease at the time retinal hemorrhage first appeared. The group included fifty-five patients with onset of diabetes before the age of twenty. These fell into three subgroups of roughly equal size with glycosuria under excellent, fair and poor control, respectively. Retinopathy developed after ten to thirteen years in all the patients without relation to control, and there was little difference between the subgroups in the incidence of hypertension and no difference in arterial calcification. Albuminuria, however, occurred twice as frequently in those poorly controlled as in those very well controlled. No evidence that poor control favors or good control protects against vascular disease was found by Bell[3] for arteriosclerosis, Wagener, Dry and Wilder[53] for retinopathy, Henderson, Sprague and Wagener[14] for intercapillary glomerulosclerosis or Lundbaek[47] for all lesions except retinopathy.

Those who take the opposite view offer impressive evidence of a clinical nature. Among the 200 young patients of White and Waskow,[4] diabetic coma, certainly the epitome of poor control, had occurred in 17 per cent of those without degenerative lesions, in 38 per cent of those moderately affected and in 74 per cent of those incapacitated. A lower incidence of retinopathy in well controlled as opposed to poorly controlled patients was found by Spoont and his associates,[58] by Lundbaek[47] and by Jackson et al.,[59] the latter demonstrating also a smaller proportion of arterial calcification. Respecting "nephropathy," Root, Pote and Frehner[60] found the incidence of this condition among 451 patients with diabetes of ten to

thirty-four years' duration to be zero (eleven cases) for those with excellent control, 2 per cent (fifty cases) with good control, 17 per cent (ninety cases) with fair control and 28 per cent (298 cases) with poor control.

TABLE III
RETINOPATHY IN 189 PATIENTS WITH DIABETES OF TWENTY TO TWENTY-NINE YEARS' DURATION*

Degree of Control	Number of Cases	Per cent of Cases	
		No, or Slight, Retinopathy	Moderate, Marked or Extreme Retinopathy
Good.....	32	76	24
Fair.....	41	52	39
Poor.....	116	33	67

* From Root, Pote and Frehner.[60]

These authors carried out similar investigations of retinopathy. Table III, which summarizes their results, deserves special attention. It shows that of patients under good control retinopathy was absent or minimal in 76 per cent and moderate, marked or extreme in 24 per cent, while of those under poor control retinal lesions were absent or minimal in 33 per cent and moderate, marked or extreme in 67 per cent. Granted the difficulties, mentioned previously, of adequately evaluating control, it is probable that these workers have done it as well as is presently possible. Their data show clearly that a high incidence of severe retinopathy is associated with poor levels of control and a low incidence with good levels. However, they show just as clearly that one-fourth of the patients with good control had serious retinal damage and one-third with poor control had almost none despite very long-standing diabetes. These figures may be taken as representing fairly the relationship of control to the occurrence of vascular disease. They strongly suggest that the maintenance of a sugar-free urine will minimize the frequency and severity of vascular disease but that this alone is not sufficient in all cases. They suggest also that there is between individuals an inherent difference in the ability of the blood vessels to withstand insults of approximately equal degree and duration.

Adrenal and Pituitary Hormones. The capillary lesions of the retina and glomerulus, as has already been indicated, have much in common. Their concurrence, while not quite universal, is extremely frequent, aneurysmal dilatations have been described in both, and both apparently contain mucopolysaccharides as revealed by staining reactions. It is therefore natural to suspect that they have a similar pathogenesis. A number of recent observations have tended to implicate adrenocortical hormones or their pituitary trophic substances. Several cases have been reported[61–63] in which diabetic retinopathy first appeared or became much worse during pregnancy and in some instances subsided after delivery, suggesting that some disturbance of steroid metabolism may be involved. In 1952 Rich[64] described lesions resembling the Kimmelstiel-Wilson type in a non-diabetic subject who had received large doses of corticotropin for long periods of time. In that same year Becker,[63] working with Friedenwald, reported the appearance of similar renal lesions, as well as retinal microaneurysms, in alloxan-diabetic rabbits treated with corticotropin. Alloxan alone was ineffectual. These results have been confirmed in part by Bloodworth and Hamwi[65] who demonstrated both nodular and diffuse glomerular lesions in normal and alloxanized rabbits treated with cortisone. The lesions, however, tended to disappear after six weeks even though treatment was continued. These authors make no statement about retinopathy. Extending his investigations of the adrenal glands, Becker[66] has found that diabetic patients with retinopathy exhibit heavier adrenal weights with more cortical vacuolization, excrete more oxysteroids in the urine and more consistently show a normal eosinopenic response to corticotropin than do patients without retinopathy.

In 1953 Poulsen[67] reported the now famous case of a woman who had acquired diabetes in 1924 at the age of nine years. In 1945, at the age of thirty, diabetic retinopathy was found. In the same year a pregnancy, resulting in a stillborn fetus, was followed by severe uterine hemorrhage with the subsequent development of Sheehan's syndrome. By 1949 the retinopathy had diminished and by 1951 it had disappeared.

It is not surprising that the sum total of these observations has led to attempts to ameliorate diabetic vascular disease by removal of the pituitary and adrenal glands. A series of hypophysectomies reported in 1953 by Luft and Olivecrona[68] included four cases of juvenile diabetes, three with advanced vascular disease.

Postoperative survival was brief in three patients, however, and no significant data respecting vascular disease are available in the fourth. In 1954 Kinsell and his co-workers[69] published an account of four patients with diabetes in whom hypophysectomy had been performed for severe renal and retinal disease. A number of interesting and expected metabolic results ensued but even one year later, after eighteen months' observation in some cases, the authors were unable to say that any unequivocal, permanent improvement in vascular status had taken place.[70] Also in 1954, Wortham and Headstream[71] reported on bilateral adrenalectomy in seven similar cases. Of these, two are said to have shown minor reversals of retinal changes and diminished blood pressure, proteinuria, azotemia and edema; in three cases the process was considered arrested; and two patients experienced progressive renal failure terminating in death from vascular accidents. Taken together, the results of these surgical procedures are not impressive as of this date.

It might be expected that examination of the retina in patients with either acromegaly or Cushing's syndrome and diabetes would be revealing. Only two such studies are available. Of twenty-one patients with acromegaly and diabetes, McCullagh and Alivisatos[74] found diabetic retinopathy in three after eight, five and two years of known diabetes, respectively; and of twenty-one patients with Cushing's syndrome and diabetes, diabetic retinopathy appeared in two after nine and eight years of known diabetes, respectively. Ricketts[75] found diabetic retinopathy in two of three patients with acromegaly and diabetes, and in none of seven patients with Cushing's syndrome and diabetes. Thus diabetic retinal disease is apparently infrequent in combination with hyperactivity of either the anterior pituitary or adrenal cortex, although it has occurred unusually early in the course of diabetes in some of the patients who showed it. Comparable investigations of hypopituitarism, with the exception of Poulsen's,[67] and of Addison's disease do not seem to have been published.

It is of the greatest importance that a method of experimentally producing capillary lesions resembling those of human diabetes has finally been discovered. It is still quite uncertain, however, to what degree, if any, adrenocortical substances are responsible for these lesions in man. The evidence adduced by Becker[66] is at this point only suggestive. The results of determinations of urinary steroids in diabetes are conflicting,[66,72,73] and there certainly is no reason to believe on clinical grounds that the long-term diabetic patient is suffering from adrenal hyperactivity. It may be that the role of the adrenal is a permissive one, merely allowing the development of lesions whose primary etiology lies in other areas.

BIOCHEMICAL FACTORS

Aside from any consideration of endocrine influences, the fact that the incidence and degree of vascular lesions are clearly related to the duration of diabetes, and probably to its severity and control, suggests that there may be some associated biochemical abnormality which, acting over long periods of time and with considerable intensity, favors, if it does not determine, the development of such lesions. The obvious abnormalities are hyperglycemia and hyperlipemia.

Hyperglycemia. No one has ever seriously proposed that concentrations of blood sugar, such as prevail in long-term diabetes, are in themselves injurious to intimal tissue, although it has been postulated without proof that extreme fluctuations may be harmful. It is possible, however, that some factor related to hyperglycemia may be implicated. In this connection attention has been directed to the mucopolysaccharides because of their presence in both retinal microaneurysms and the glomerular lesions of Kimmelstiel and Wilson. Earlier work in diabetic patients which indicated a direct relationship between levels of blood glucose and glucosamine,[76] a component of mucopolysaccharides, has not been conclusive.[77] Berkman and others[77] nevertheless demonstrated higher levels of protein-bound polysaccharides and glucosamine in the serum of patients with clinically apparent vascular disease than in those without. Nielsen and Poulsen[78] found increased concentrations of similar compounds in the blood of diabetic patients without complications as well as in those with renal disease. These results were confirmed by Keiding and Tuller[79] who found in addition that levels in patients with "nephropathy" were higher than in those showing degenerative lesions without nephropathy. Gilliland et al.[80] report some parallelism between polysaccharide levels and the degree of vascular disease but also some increase in levels before such disease was clinically manifest. Investigations of the urinary excretion of these substances

have shown no correlation with diabetes or its complications.[81] In view of the increased concentrations of serum mucopolysaccharides that are known to occur in conditions characterized by tissue destruction and repair,[77] it is possible that the higher values observed in diabetes may be the result of degenerative disease, either patent or occult, rather than its cause. Regardless of such cautionary considerations, the subject deserves the further study that it is certain to receive.

*Hyperlipemia.** Except for the presence of fat in a considerable proportion of the lesions of intercapillary glomerulosclerosis,[11] there is little reason to believe that hyperlipemia has any etiologic significance for that condition or for diabetic retinopathy. Its relationship to atherosclerosis in general has been the subject of a vast body of literature. The tendency of diabetic individuals toward both abnormal fat metabolism and atherosclerosis has often been used as an argument favoring a causal relationship between the two even in non-diabetic persons. It is pertinent to inquire, however, as to the frequency of high levels of serum lipids in the ordinary diabetic patient. "For our present purposes, we are not interested in the characteristic lipemia of the untreated diabetic or of the diabetic in acidosis. If the lipids have any significance for vascular disease, it must be by virtue of the levels which prevail in the treated diabetic over a long period of years. The literature contains surprisingly few reports on this subject, and those that we have are not only conflicting but are based on analyses made at only a single point in time.†

"Chaikoff and others[83] compared serum cholesterol, phospholipids and fatty acids in twenty-three normal children and twenty-six children with diabetes under good control with diet and insulin. They found no appreciable difference between the two groups, nor did the duration of diabetes or the daily insulin requirement (10 to 68 units) have any relation to the cholesterol levels. Man and Peters[84] found the serum cholesterol normal or below in 64 per cent of seventy-nine diabetics not suffering from dehydration or acidosis. Of nineteen patients who showed rather marked hypercholesterolemia, sixteen had complicating conditions such as cirrhosis or nephritis which in themselves might have explained the high cholesterol levels. Among the uncomplicated cases the cholesterol did not exceed 304 mg. per cent—certainly not a striking figure. Pomeranze and Kunkel[85] recently examined two hundred seventy-three diabetics in various clinics in New York City. Almost exactly half had total lipids above 750 mg. per cent (the upper limit of normal) while the other half had levels below this." Among 614 diabetic patients, Barach and Lowy[86] found 42 per cent with normal and 58 per cent with high cholesterol values. Lundbaek[47] found the average serum cholesterol concentration in 151 long-term patients to be only 256 mg. per 100 ml. In patients below the age of forty, values were from 30 to 40 mg. higher in diabetic than in normal persons, but above the age of forty there was no significant difference.

"These data suggest that in children with well controlled diabetes there is little if any increase in blood lipids but that among adults, with diabetes of varying severity and under varying degrees of control, the lipids are elevated, although not markedly so, in 35 to 58 per cent of the cases.

"Respecting lipoproteins in diabetes, Barach and Lowy[86] report the Sf 12–20 fraction above 50 mg. per cent in 33 per cent of males and 43 per cent of females. Hanig and Lauffer[87] were unable to find any significant difference between normal persons and diabetics. The data of Keiding and associates[88] would justify a similar conclusion, although they do not state it in so many words. Among the diabetics themselves, Sf 12–20 levels tended to be higher in patients under poor control than in those under good control.

"The next question is whether the diabetics who do have hyperlipemia are the ones who also have vascular disease. Among the cases studied by the New York group,[85] 78 per cent of the patients with hyperlipemia had severe atherosclerosis as shown by electrocardiographic evidence of coronary disease, calcified vessels or retinal arteriosclerosis. Twenty-two per cent were classed as having moderate atherosclerosis or none. On the other hand, of the patients who had normal serum lipids about 40 per cent had severe atherosclerosis and 60 per cent had a moderate amount or none. We are left, then, with the dilemma of what caused the vascular disease in the 40 per cent without hyperlipemia.

"Referring again to the lipoproteins, Barach[86]

* The ensuing passages indicated by quotation marks are taken from an earlier review of this subject by the author.[82]

† See footnote, page 942.

found the Sf 12–20 fraction elevated in 45 per cent of one hundred sixty-two diabetics with vascular calcification. This is not significantly different from the incidence of elevated Sf 12–20 levels in the entire group of diabetics. Essentially the same results were reported by Keiding,[88] with the additional surprising finding that lipoproteins were distinctly increased in patients with retinopathy. It is not stated how many of the patients with retinopathy also had the Kimmelstiel-Wilson lesion—a condition in which the Sf 12–20 levels are almost uniformly high."[89]

Finally, studies of quite a different sort by Hirsch and his colleagues[90] have shown that in diabetic persons the esterified fatty acids of the blood, in contrast to cholesterol and lipoproteins, rise abruptly after a fat meal and that the increase is greater during periods of hyperglycemia than during normoglycemic periods. The fasting levels, moreover, when determined day after day in patients with unstable diabetes, rise and fall *pari passu* with the fasting blood glucose. If it were established that arterial disease is in fact induced by hyperlipemia, these observations would provide a powerful argument for strict control of the blood sugar.

To summarize, hyperlipemia is not a prominent feature of treated diabetes. It is present to a greater extent in patients with poorly controlled than in those with well controlled glycosuria. Studies of its relation to vascular disease have yielded inconsistent results. Respecting atherosclerosis, there are no available data on serial determinations of serum lipids in the same diabetic patients over a period of years.* Single analyses suggest some association between elevated lipid concentrations and the presence of atherosclerosis, but such association is lacking in a large number of cases. Even a good correlation would not prove a cause and effect relationship. Patients with presumed intercapillary glomerulosclerosis have higher lipid levels than those without, but lipids are also elevated in non-diabetic persons with other forms of nephritis. It is probable that the hyperlipemia of some patients with retinopathy is explained by the presence of renal disease.

* In a study of intercapillary glomerulosclerosis, in which data for atherosclerosis are not given, Mann, Gardner and Root[91] report that in patients for whom information on cholesterol was available, values were normal when the patients first came under treatment and remained so for the first ten years of diabetes, rising to abnormal levels only with the onset of symptoms of nephritis.

SUMMARY

Diabetes, or some factor related to it, markedly hastens the onset and increases the frequency and severity of arterial and arteriolar sclerosis. In addition, it gives rise to capillary lesions of the renal glomeruli and retina that belong almost exclusively to the diabetic state. Rates of death and disability from these causes are increasing as diabetic patients live longer.

Diabetes increases susceptibility to atherosclerosis to a greater extent in women than in men, and the former have a higher incidence of intercapillary glomerulosclerosis but not of retinopathy.

Although instances of vascular disease are observed in greater numbers among older patients, its incidence and seriousness in younger patients with long-standing diabetes are striking. Duration of diabetes stands out as the most important single factor, and its effects are greater in younger than in older persons.

The parts played by severity and control of diabetes are still in dispute. The best evidence indicates that the highest frequency of advanced lesions occurs in severely diabetic patients with prolonged, heavy glycosuria. It is clear, however, that some patients with mild, well controlled diabetes have manifest vascular lesions while their opposite numbers seem to escape. These inconsistencies lend support to the concept, as yet incapable of proof, that hereditary tendencies may be as important as diabetes itself.

The experimental production of both glomerular and retinal lesions with cortisone and corticotropin strengthens the possibility that they have a common pathogenesis in man and raises the question of whether their clinical occurrence is related to adrenal hyperactivity. Evidence on this point is contradictory.

Mucopolysaccharides are increased in the blood of diabetic patients with vascular, especially renal, disease but to some extent in those without it and may be related to the pathogenesis of retinopathy and intercapillary glomerulosclerosis.

Hyperlipemia in the treated diabetic patient is neither so frequent nor so marked as has been commonly supposed. To the extent that it does exist its association with atherosclerosis is loose and inconstant and in many cases is lacking entirely. Elevated lipid levels in intercapillary glomerulosclerosis are probably a result rather than a cause of the disease.

The etiology and pathogenesis of diabetic vascular disease remain obscure.

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Clinico-pathologic Conference

Hypertension, Abdominal Pain and Nausea, Syncope and Shock

STENOGRAPHIC reports, edited by Albert I. Mendeloff, M.D. and David E. Smith, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

PATIENT H. H., (History No. 230473), a forty-six year old white married housewife, was admitted to the Barnes Hospital for the first time on December 20, 1953, with a chief complaint of "blackout spells" two hours prior to admission.

The patient was said to have had hypertension during both of her pregnancies. However, blood pressure returned to normal between pregnancies. Two years prior to admission she had a hysterectomy, appendectomy and oophorectomy because of persistent metrorrhagia. At that time she was found to have sustained hypertension and cardiac enlargement. Following this operation she had repeated episodes of hot flashes for which she was given oral hormones intermittently without relief. She had occasional headaches and episodes of epistaxis. About six to eight weeks prior to admission she began to have attacks of irregular beating of her heart and an uncomfortable feeling of fullness in her head, "like everything was rushing into my head." She would feel as if she were going to faint but never did. She also noted ringing in her ears and occasionally an unsteady feeling. On the day prior to admission she had such an episode which passed off without sequelae. Because of her palpitation and the finding by her attending physician several weeks prior to admission of ventricular premature contractions, she was placed on quinidine, 0.2 gm. four times a day, which did not seem to affect the frequency of occurrence of her palpitations. A blood pressure reading in her physician's office several weeks prior to admission was in the range of 240 to 250/110 to 120.

Two hours prior to admission, while lying down, a feeling of fullness in her head developed and she became unconscious. There were no other premonitory symptoms. She did not convulse, bite her tongue or injure herself and there was no incontinence of urine or feces. She did, however, stop breathing and a neighbor began to breathe into her mouth. Police were called and an inhalator was used. Her attending physician arrived approximately thirty-five minutes after the onset of her unconsciousness. He found her in "pulmonary edema" with a very weak pulse. She was given 150 mg. of aminophylline intravenously. Prior to this episode the patient had never had any symptoms of cardiac decompensation such as orthopnea, paroxysmal nocturnal dyspnea, dependent edema, or dyspnea on slight to moderate exertion.

Her past history was notable in that eight years prior to admission she had episodes of nausea without pain and was said to have had a peptic ulcer which was treated with a bland diet, pills, and non-absorbable antacids, following which her symptoms subsided. Several months prior to admission she had an episode of epigastric tightness and distress, most marked in the right upper quadrant. Roentgen examinations of the gastrointestinal tract, gallbladder and kidneys were said to be negative. She was told several months prior to admission that her episode of abdominal distress was probably a recurrence of her ulcer, and she was advised to follow a bland diet again. One stool was found to contain blood but she had never noted gross blood or melena although she had looked for it. There was no jaundice.

The family history disclosed that her mother died at the age of seventy and her father at the age of sixty-nine, both of strokes.

Physical examination at the time of admission revealed the patient's temperature to be 37.1°c.;

pulse, 100; respirations, 16 and blood pressure, 150/100. The patient was apprehensive, slightly cyanotic and confused. She was well developed, well nourished, acutely ill and coughing frequently. There was no lymphadenopathy. Examination of the eyes revealed normal extraocular movements. The pupils were round and equal and reacted to light. Examination of the fundi revealed slight arteriolar-venous nicking without papilledema, hemorrhages or exudates. On the right posterior oral pharyngeal wall were yellow punctate lesions, each of which was surrounded by an area of erythema. Her neck was supple and the thyroid was not palpable. There were a few fine rales at the left lung base. The heart was enlarged to the anterior axillary line with a well localized point of maximum impulse in the fifth intercostal space. There was a harsh, grade II systolic murmur best heard in the region of the pulmonic area. There was a regular diastolic gallop rhythm at the apex. The sounds were of poor quality. No masses or organs were palpable on abdominal examination. Examination of the extremities revealed slight cyanosis of the nail beds. There was no edema or clubbing. Pedal pulses were present. The remainder of the physical examination, including the neurologic examination, was not remarkable.

The laboratory data were as follows: red blood cell count, 6,320,000 per cu. mm.; hemoglobin, 16.8 gm. per cent; white blood cell count, 21,050; differential count: stab forms, 3 per cent; segmented forms, 75 per cent; lymphocytes, 19 per cent; monocytes, 3 per cent. Urinalysis: specific gravity, 1.008; pH, 7.0; protein, 2+. Serologic test for syphilis: negative. Sputum culture: moderately heavy growth of neisseria, slight growth of staphylococci and alpha hemolytic streptococci. Electrocardiogram: left ventricular strain, question of subendocardial myocardial injury, sinus tachycardia and quinidine effect. Blood chemistry: non-protein nitrogen, 22 mg. per cent; fasting sugar, 106 mg. per cent; two-hour postprandial sugar, 96 mg. per cent; carbon dioxide combining power, 31 mM/L.; prothrombin level, 80 per cent of normal. Erythrocyte sedimentation rate, 4 mm. per hour, corrected.

The patient was given oxygen by nasal catheter, following which there was an immediate disappearance of cyanosis and an increase in alertness; nasal oxygen was continued for four days. An electrocardiogram on the second hospital day showed no change from the one taken on the first. Venous pressure on the second hospital day was 45 mm. of water; arm-to-tongue circulation time was twenty-two seconds. On the third hospital day the patient's lungs were clear to auscultation. An electrocardiogram on the fourth hospital day showed T wave inversion across the precordium compatible with anterior myocardial ischemia or pericarditis; subendocardial myocardial injury was no longer evident, and there was less evidence of quinidine effect. A repeat blood count on the fourth hospital day was as follows: red blood cell count, 4,690,000; hemoglobin, 13.5 gm. per cent; white blood cell count, 9,300; differential count: eosinophils, 2; stab form, 1; segmented forms, 56; lymphocytes, 40; monocyte, 1. Red blood cells showed slight hypochromasia. On the fifth hospital day a pericardial friction rub was heard in the second and third left intercostal spaces along the left sternal border. This rub persisted to the fifteenth hospital day. During this time the patient's lungs were otherwise clear on physical examination. The systolic gallop rhythm heard at the apex of the heart persisted. An electrocardiogram on the eleventh hospital day was interpreted as showing reappearance of subendocardial myocardial injury, left ventricular strain and sinus tachycardia. Subsequent electrocardiograms revealed resolving subendocardial myocardial injury, then left ventricular strain with a question of anterior myocardial injury, then left ventricular strain with a question of anterior myocardial ischemia, and finally only an abnormal form of ventricular complex on the fifty-ninth hospital day. Quinidine in doses of 0.2 gm. four times a day and later three times a day was continued throughout her hospitalization.

During the first week of the patient's hospital course her blood pressure ranged from a high of 180/120 to a low of 130/90. On the third hospital day apresoline® in doses of 25 mg. three times a day was started. This dose was gradually increased and on the eleventh hospital day hexamethonium chloride was administered. The patient's blood pressure continued to fluctuate considerably until approximately the last two weeks of hospitalization when her blood pressure stabilized in the approximate range of 160 to 170/90 to 100. The patient remained on absolute bed rest until the twenty-eighth hospital day when gradually increasing ambulation was begun. Repeated determinations of the blood

non-protein nitrogen were within normal limits. The erythrocyte sedimentation rate was normal until the thirty-second hospital day when it was 23 mm. per hour; on the forty-second hospital day it was 28 mm. per hour. On the fifty-second hospital day the phenolsulfonphthalein excretion was 24 per cent in fifteen minutes and 67 per cent in two hours. Urine concentration test at eighteen hours produced a specific gravity of 1.012. The patient tolerated her medication quite well and was discharged on February 18, 1954, on doses of apresoline, 100 mg. every four hours (except at 2 A.M. when the dose was to be omitted) and hexamethonium every four hours, the dose not to exceed 200 mg. every four hours according to the blood pressure which was to be determined by her husband. She was also given a sedative and a non-absorbable antacid.

The patient continued her antihypertensive therapy and had no untoward complaints until one week prior to her second admission when she first noted epigastric "fullness and tightness" and a "cold" which she treated with aspirin, bicarbonate of soda and a bland diet. She had no fever or sore throat. She was admitted on September 24, 1954.

Physical examination on this admission recorded the patient's temperature to be 36.6°C.; pulse, 88; respirations, 18 and blood pressure 180/120. She appeared chronically ill and complained of abdominal discomfort. Examination of the abdomen revealed diffuse tenderness most marked in the mid-epigastrium without muscle guarding, rigidity or rebound tenderness. Bowel sounds were normal. In the right upper quadrant there was felt to be a tender cystic mass which moved with respirations. The remainder of the physical examination was otherwise as on her previous admission.

The laboratory data were as follows: hemoglobin, 14.0 gm. per cent; white blood cell count, 12,650; differential count: segmented forms, 69 per cent; lymphocytes, 28 per cent, monocytes, 3 per cent. Urinalysis: specific gravity, 1.017; pH, 7.5; protein, 1+; sugar, negative; microscopic examination: two to four white blood cells per high power field. Stool examination: guaiac-negative. Blood chemistry: non-protein nitrogen, 30 mg. per cent; fasting sugar 71 mg. per cent; cholesterol, 262 mg. per cent; albumin, 4.3 gm. per cent; globulin, 2.7 gm. per cent; alkaline phosphatase, 8.1 Bodansky units; total bilirubin, less than 0.8 gm. per cent; icterus index, 21.7 units; cephalin cholesterol flocculation, negative; thymol turbidity, 0.9 units. An electrocardiogram on admission showed a full auriculoventricular conduction time but was otherwise within normal limits. Roentgen examination of the chest revealed bilateral upper lobe fibrocalcific tuberculosis. Routine cholecystograms revealed cholelithiasis; the gallbladder was well visualized.

The patient vomited food and liquids on several occasions but her symptoms gradually subsided. It was felt on subsequent examinations that the "mass" in the right upper quadrant was only rectus muscle. Blood chemical determinations done on the fourth hospital day were as follows: alkaline phosphatase, 3.2 Bodansky units; amylase, 200 units; prothrombin level, 80 per cent of normal. Her blood pressure was within normal limits through most of her hospital stay with only an occasional rise to hypertensive levels. She was discharged asymptomatic on the seventh hospital day, September 30, 1954.

The patient had apparently continued her antihypertensive therapy, varying the dose according to the blood pressure. She took only 125 mg. of hexamethonium chloride on the day prior to admission; she had taken none on the day of admission. About 6:30 A.M. on October 11, 1954, she began to have generalized cramping abdominal pain, most marked in the suprapubic area, associated with vomiting on one occasion and gagging. The cramping was also associated with the desire to defecate but she was unable to do so. She gave herself a small enema without recovery of the enema fluid. There had been no bowel movement on the day prior to admission. However, there was a normal bowel movement the day before that. The cramping continued intermittently all during the day of admission.

On this admission the patient's temperature was 37.5°C.; pulse, 104; respirations, 22 and blood pressure 120/80. The patient appeared chronically ill and preferred to have her knees and legs flexed while lying on her side. On abdominal examination a mass thought to be the sigmoid colon full of feces was felt in the left lower quadrant. In the right lower quadrant there was tenderness without rebound. Bowel sounds were hypoactive. There was no hyperperistalsis associated with cramping. The remainder of the physical findings had not changed from the last examination on the previous admission.

The laboratory data were as follows: hemoglobin, 16.8 gm. per cent; white blood cell count, 22,525; differential count, stab forms, 12 per cent; segmented forms, 80 per cent; lymphocytes, 7 per cent; monocytes, 1 per cent. Urinalysis: specific gravity, 1.010; pH, 7.4; protein, negative; sugar, negative; microscopic examination: occasional hyalin cast, occasional epithelial cell, few phosphate crystals; bilirubin, negative. Stool: guaiac-negative. Urine culture: few colonies of coagulase-negative Staphylococcus albus. Blood chemistry: non-protein nitrogen 33 mg. per cent; fasting sugar, 131 mg. per cent; amylase, 89 units; cephalin cholesterol flocculation, negative; thymol turbidity, 1.5 units; total serum bilirubin, less than 0.8 mg. per cent; albumin, 5.2 gm. per cent; globulin, 3 gm. per cent; alkaline phosphatase, 8.4 Bodansky units; calcium, 11.3 mg. per cent; phosphorus, 5.9 mg. per cent; sodium, 137.9 mEq./L.; potassium, 3 mEq./L.; chloride, 94 mEq./L.; carbon dioxide combining power, 20.8 mM/L. An L.E. cell preparation was negative. Blood culture: no growth. An electrocardiogram was interpreted as follows: "The marked positional changes with the tall R wave in AVR point strongly toward a pulmonary embolus." Posterolateral myocardial infarction, indeterminate heart position with marked clockwise rotation and sinus tachycardia were also diagnosed.

The patient's blood pressure after admission was extremely labile, reaching a height of 220/140 three hours after admission, but early on the morning of the second hospital day it began to fall. Concomitant with the fall in blood pressure her temperature began to rise. Ten milligrams of vasoxyl® given intramuscularly produced only a transient rise in her blood pressure with a prompt return to a level in the range of 70 systolic, the diastolic pressure being unobtainable. The patient continued to have abdominal pain and vomited guaiac-positive material. She was given intravenous fluids to which nor-epinephrine was added on the morning of the second hospital day. Her blood pressure responded only transiently. Her level of consciousness decreased progressively; on the morning of her second hospital day she became totally unconscious. Despite the addition of increasing amounts of nor-epinephrine to the intravenous fluids, her blood pressure continued to fall. Her temperature increased to 42.5°C. She was placed in a refrigerated oxygen tent and icebags were placed on her abdomen and axillas. Her respirations became increasingly more gasping and on the morning of her second hospital day they ceased entirely, her heart sounds could not be heard and her blood pressure was unobtainable. An intracardiac injection of 1 cc. of 1:1000 epinephrine failed to produce a response. Blood chemical determinations obtained prior to her death on the second hospital day were as follows: non-protein nitrogen, 60 mg. per cent; chloride, 90 mEq./L.; carbon dioxide combining power, 15.6 mM/L. She died on October 12, 1954.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: We should perhaps first consider what type of hypertension this patient had. She was said to have had hypertension during her two pregnancies, with a return to normal blood pressure between pregnancies. There was no information in the chart as to how long ago either of these pregnancies occurred. In 1951, two years prior to her first admission to the Barnes Hospital, she was found to have sustained hypertension when she was hospitalized for hysterectomy. Following this operation she had occasional headaches and episodes of epistaxis. From that time until twenty-four hours before her death she had a variable but definite hypertension. Several months prior to her first admission intravenous pyelograms were taken elsewhere and were said to have been normal. During her first admission to the Barnes Hospital she had 2+ proteinuria on one occasion; forty-eight hours later there was no proteinuria. Her blood nonprotein nitrogen concentration was normal then, and remained so until she went into shock twenty-four hours before her death. The history at the time of her first admission stated that the patient had been emotionally very unstable and subject to anxiety attacks since her hysterectomy; these were more severe after her mother died of a cerebral hemorrhage in February, 1953. We have no information about the patient's emotional status prior to the onset of her hypertension. Both the patient's mother and father are stated to have died of strokes. Dr. Schroeder, do you think the hypertension we are dealing with here is essential hypertension? That was the clinical diagnosis. Do you see any reason to doubt it?

DR. HENRY A. SCHROEDER: I never make a diagnosis of essential hypertension. It is very

hard to tell from the protocol just what kind she had. I would suspect that she had what we might call neurogenic hypertension, associated with overactivity of the autonomic nervous system, or she may even have had an endocrine component. She had had a hysterectomy and had hypertension with her pregnancies; it is possible that she may have had adrenal hyperplasia or even a small adenoma.

DR. REINHARD: Does anybody have anything to add to this? We can agree with Dr. Schroeder that the data available do not permit us to define the hypertension much more precisely.

DR. EDWARD MASSIE: Dr. Reinhard, I would like to ask Dr. Schroeder if this patient could have had a pheochromocytoma? She was young, she had arrhythmias, abdominal pain and many other features described in patients with that disease; perhaps we have information about her only at the stage of her disease when the blood pressure was persistently elevated.

DR. SCHROEDER: That is a very good suggestion, Dr. Massie. The history of hot flashes and palpitation could be consistent with either neurogenic hypertension or with pheochromocytoma. I suspect that when one gives a ganglionic blocking agent to a patient with pheochromocytoma the blood pressure does not come down. As a matter of fact, these drugs may make the tumor discharge. I have not had that experience myself but it has been reported.

DR. REINHARD: In view of the fact that no tests were performed to make the diagnosis of pheochromocytoma, I believe we can only say that this is a possibility here. Let us consider the blackout spells which were the immediate cause of this patient's first admission. Six to eight weeks prior to admission she began to have attacks of irregular beating of her heart, an uncomfortable feeling of fullness in her head, a ringing in her ears, and a feeling that she was going to faint although she never did. One day prior to admission she had an episode of this sort which passed off without any sequelae. Because of her palpitation and the finding by her attending physician several weeks prior to admission of ventricular premature contractions, she was placed on quinidine, .2 gm. four times a day; this did not seem to affect the frequency or severity of the palpitations. Blood pressure readings in her physician's office several weeks prior to admission were 240 or 250 systolic and 110 or 120 diastolic on several examinations. Two hours prior to admission, while she was lying quietly on a divan, a feeling of fullness in her head developed and she became unconscious. She did not convulse, did not bite her tongue and did not injure herself. There was no incontinence of urine or feces. She did, however, stop breathing, and a neighbor began to breathe into her mouth. Police were called and an inhalator was used. Her attending physician arrived approximately thirty-five minutes after the onset of her unconsciousness. He found the patient to be in pulmonary edema with a very weak pulse. She was given 150 mg. of aminophylline intravenously. Prior to this episode she had never had any symptoms of cardiac decompensation. The house officers who admitted this patient to the hospital thought this attack of unconsciousness was due either to a sudden cardiac arrhythmia or to decreased cerebral blood flow due to the cardiac decompensation. However, although the heart was enlarged, the liver was not; there was no edema and there was no very convincing evidence of cardiac decompensation. Dr. Massie, I believe you have studied this patient's electrocardiograms. Would you tell us what the electrocardiograms on this admission showed, and then explain to us what you think these attacks of palpitation, as well as the single episode of unconsciousness, might have been due to. Were these due to the same cause or to different causes?

DR. MASSIE: Looking over the eleven electrocardiograms I would say this patient suffered successively myocardial ischemia, left ventricular strain, myocardial injury and then subsequently developed a fairly normal record. In the first set of tracings ischemia is seen primarily, and I can go no further than to blame coronary insufficiency as the cause of the electrocardiographic findings. I would not necessarily incriminate the heart as the cause of the blackout spell.

DR. REINHARD: This patient did have a pericardial friction rub from the fifth to the fifteenth hospital day. How do you account for this?

DR. MASSIE: I do not believe that this patient had a myocardial infarction. Therefore, I would not make a diagnosis of pericarditis resulting from myocardial injury and infarction. I cannot explain why she had pericarditis; the electrocardiogram is not very impressive, and I wonder whether she did have it. The friction rub may have been something else. I presume it was not a pleural–pericardial friction rub?

DR. REINHARD: Dr. Wilson, was this a friction rub?

DR. KEITH WILSON: I thought it was a pericardial friction rub.

DR. MASSIE: In that case, I cannot explain it on the basis of what I see in the protocol or the electrocardiogram. We would have to ascribe it to an arrhythmia. In these eleven electrocardiograms we have no arrhythmia. I do not know how cerebral syncope occurs in cardiac disease without shock, without acute infarctions or without arrhythmias.

DR. REINHARD: Dr. Wilson, did this patient ever have an arrhythmia when you saw her in the office?

DR. WILSON: No, but I saw her only about two weeks before she entered the hospital.

DR. JOHN SMITH: The remark she made that there was a feeling of fullness in her head "like everything was rushing into my head" is extremely reminiscent of venous congestion, and one wonders whether or not these were episodes of sudden vasodilatation which were occasioned by some type of vagal depressor response. One wonders whether the carotid sinus might have been active in a patient like this, there being particularly a cerebral type of response; the palpitation therefore would follow in natural sequence to it.

DR. REINHARD: We may be comforted by the thought that there may be no pathologic changes to account for this attack of unconsciousness, but perhaps we will not have to apologize for that. We certainly cannot explain this adequately. Let us proceed now to the patient's second hospital admission seven months later in September, 1954. The chief complaint that brought the patient to the hospital this time was epigastric fullness and a tightness of the epigastrium, without any real pain, of one week's duration. The patient also had a cold. She apparently had been nauseous and had vomited several times during the two days before admission. Eight or nine years prior to this, at the age of thirty-eight, the patient had several episodes of nausea without pain. Apparently no x-rays were taken at that time, but she was told that she had a peptic ulcer and was treated with antacids and a diet following which her symptoms subsided. In the fall of 1954 she had episodes of epigastric tightness and right upper quadrant distress at which time gastrointestinal x-rays were obtained elsewhere; these were said to have been normal. We have not seen them. However, she was told that she probably had a recurrence of her ulcer and was put on a bland diet. She never had gross blood in her stools or melena, but one stool was guaiac positive at the time of her second admission to the hospital or just before it. An electrocardiogram taken on the day of admission to the hospital showed prolonged conduction but was otherwise not remarkable. Six stool specimens examined while the patient was in the hospital were all guaiac-negative. I think the protocol mentions only one. This is a good time to see the x-rays, as no films were taken of this patient except during the second hospital admission.

DR. WILLIAM B. SEAMAN: On the first day of the second hospital admission the patient had examinations of the chest and abdomen. The examination of the chest showed a cardiac shadow that lay within the upper limits of normal. It might perhaps have been slightly enlarged, but without previous films it was impossible to say. She had calcified peritracheal nodes, a small focal parenchymal calcification lying in the subpleural position in the apex of the lower lobe, and in both upper lobes there were small focal areas of calcification. These all were compatible with an old calcified tuberculosis. The lungs were otherwise clear. The examination of the abdomen revealed a gas pattern in the intestinal tract which was not remarkable. The liver shadow extended down a little below the iliac crest; there was no evidence of calcified gallstones. There were a few calcifications overlying the kidney on the right side, but in the lateral view they seemed to lie within the substance of the liver rather than in the kidneys. Kidney shadows were normal in size. Three days later a cholecystogram showed normal visualization of the gallbladder, but overlying its shadow were some radiolucent areas which could have been stones.

DR. REINHARD: Dr. Wilson, this was your patient. Did you think the symptoms at the time of her second admission to the hospital were due to cholelithiasis or cholecystitis? That was the favorite clinical diagnosis while the patient was in the hospital. You will recall that the patient had an icterus index of 21 on the day of admission to the hospital, which in the presence of gallstones would certainly suggest common duct obstruction; however, the patient was not clinically jaundiced, and the total serum bilirubin on the same day was less than 0.8 mg. per cent, so one would suspect the icterus index

was due to some other factor. What was your impression at this time?

DR. WILSON: I did not see her at that time, but apparently everyone thought she had some process going on in her abdomen, most likely in the gallbladder.

DR. REINHARD: Dr. Mendeloff, would you like to make any comments about the probable cause of this patient's symptoms at this time?

DR. ALBERT I. MENDELOFF: None of the data we have, Dr. Reinhard, would allow me to state that her symptoms were due to gallstones, if she had them, or to an ulcer.

DR. REINHARD: The alkaline phosphatase was elevated at the time of her admission to the hospital, and four days later it was back to normal; however, during the patient's terminal hospital admission the alkaline phosphatase was again elevated. Would you like to comment on possible causes for the elevated alkaline phosphatase, Dr. Shank? Is it the result of liver disease?

DR. ROBERT E. SHANK: There is really no evidence of any liver disturbance in the protocol.

DR. REINHARD: Dr. Mendeloff, how about the amylase of 200 Somogyi units on the fourth day of this hospital admission? Is this of any help?

DR. MENDELOFF: An isolated amylase value of 200 is not really abnormal. There was a later value of 89. These were the only two that were recorded on this patient, and together they do not help us very much. We do know that people with hypertension have marked changes in their pancreatic vessels, and a number of them have a certain amount of pancreatitis which is not clinically significant. This woman did have some findings which suggest that she might have had pancreato-biliary disease, and she was hypertensive, but there was little to make me believe that she had an acute pancreatitis.

DR. REINHARD: The patient died twenty-two hours after the third and final hospital admission. She had apparently continued her antihypertensive therapy, varying the dose according to the blood pressure. She took only 125 mg. of hexamethonium chloride on the day prior to admission and had taken none on the morning of admission. At about 6:30 A.M. on the day of admission she began to have generalized cramping abdominal pain most marked in the suprapubic area but involving other areas as well. This was associated with vomiting on one occasion and considerable gagging. The cramping was also associated with the desire to defecate, but the patient was unable to move her bowels. She gave herself a small enema without recovering the enema fluid. There had been no bowel movement on the day prior to admission, but there was a normal movement the day prior to that. The cramping abdominal pain continued intermittently all during the day of admission to the hospital. The significant physical findings on admission were a temperature of 37.6°C. which rose rapidly thereafter. The blood pressure fluctuated widely for four or five hours from extreme hypertensive levels to almost normal or hypotensive levels, following which she went into shock and the blood pressure went down to shock levels and stayed there in spite of therapy. The abdominal pain was less severe when the patient lay on her side with her legs flexed. There was right lower quadrant tenderness without any rebound tenderness. The comment was made repeatedly in the progress notes that in spite of the cramping pain there was no hyperperistalsis and, in fact, it was thought that the bowel sounds were rather definitely hypoactive. The lungs were clear. There was no significant hepatomegaly and there was no edema. An electrocardiogram was taken shortly before the patient's death.

DR. MASSIE: This eleventh electrocardiogram showed a remarkable change from the previous tracing of September 25, 1954, which we called a normal record; this last one revealed a marked clockwise rotation which indicates that the patient probably had had a pulmonary embolus. She may have had a posterior myocardial infarction or a lateral infarction, but in view of the marked degree of clockwise rotation I would favor the pulmonary embolus as the cause of this electrocardiographic change.

DR REINHARD: Dr. Massie, this was originally read as showing both pulmonory embolus and myocardial infarction.

DR. MASSIE: I would take away the myocardial infarction and say that pulmonary embolus was the cause of this remarkable change. With the drop in blood pressure one might think that this was a case of terminal myocardial infarction, but when you have electrical axis changes of this degree, a change from a horizontal heart position to almost a vertical heart position, I do not believe one can say the patient had infarction alone. If the patient had an infarction, she also had a pulmonary embolus, but I would prefer to hold to one diagnosis.

DR. REINHARD: Where did this embolus come from, Dr. Massie?

DR. MASSIE: This is a rather strange case. As I listen to the gastroenterologists try to explain abdominal pain here without any objective proof of gastrointestinal trouble, I am reminded of a few patients I have seen with pheochromocytoma; they had abdominal pain and cramping as this patient did. As to where the pulmonary embolus came from, I do not know; perhaps it came from the legs.

DR. REINHARD: Dr. Smith, what do you think was the cause of this patient's electrocardiographic change?

DR. SMITH: I would agree with Dr. Massie entirely; this is a picture of extreme right ventricular enlargement and dilatation probably due to acute pulmonary arterial obstruction. Now whether it was an embolus or whether there was a thrombus formed spontaneously within the pulmonary tree, I do not know. One wonders whether or not there was diffuse vascular disease of some sort going on with so many organ symptoms.

DR. MENDELOFF: In patients of this age and with this amount of vascular disease—if she really had this much over a period of time—one has to think about mesenteric thrombosis. Among the puzzling aspects of this case was the terminal temperature curve which suggested intracerebral rather than intra-abdominal disease, but I should like to have a more detailed account of her abdominal findings. Apparently this pain started low down and ended up as a low, cramping sensation. Finally, she vomited blood. This certainly would have been consistent with mesenteric thrombosis. She apparently passed no blood by rectum.

DR. REINHARD: The description of the abdominal findings is as follows: "abdomen flat and soft with doughy consistency, apparently due to minimally distended colon throughout, moderate tenderness throughout, especially in the left lower quadrant and the right lower quadrant, liver down one fingerbreadth, hypoactive bowel sounds, no rebound tenderness." I must confess that in going over this case I thought mesenteric vascular occlusion, either venous or arterial, was a diagnosis that would have to be very seriously considered. After all, emboli to the mesenteric artery often originate in the heart as a result of myocardial infarction. They may originate in the aorta; they may originate in the pulmonary vein. As far as the clinical picture was concerned, it certainly seemed to be compatible with mesenteric thrombosis. Mesenteric occlusion is characterized by abdominal pain, usually cramping or colicky. This is a symptom which this patient had. Nausea and vomiting occur and tend to be mild. They often subside after a short period of time. The patient may have constipation and diarrhea; there may be moderate distention without abdominal rigidity. Later on the picture changes into one of intestinal obstruction, vomiting of bloody material often occurs, and there may be blood in the stools. The pain becomes constant and very severe. That may have been masked in this case by the unconsciousness. Peristalsis is often hyperactive in the early phase but then becomes hypoactive, and this patient would then fit the later stage of this syndrome. Fever usually occurs, although subnormal temperatures have been reported. Leukocytosis is almost always present and may be very marked, the white count often reaching levels of thirty to forty thousand. This patient's leukocyte count was twenty-two thousand. Shock almost always occurs and usually does not respond to fluid replacement or to other medical procedures. Dr. Smith, does this appeal to you at all?

DR. SMITH: Yes, I would say it is a very reasonable course of events.

DR. JAMES OWEN: I would like to suggest the possibility of a dissecting aneurysm.

DR. REINHARD: That is another possibility we have to consider. Some of the features of this case would fit in with it. First of all, dissecting aneurysm usually occurs in patients who have long-standing hypertension. Furthermore, if the aneurysm involves the ascending aorta, it may compress or dissect along the coronary segments and produce findings compatible with myocardial infarction; however, if that were the case it would have to involve the ascending aorta, and I believe you would anticipate that the patient would have thoracic pain at least early in the process even though the pain might be abdominal later on. The clinical manifestations of a dissecting aneurysm include a sudden onset, the pain reaching maximum intensity usually very quickly, although there is great variability in the pain. Moderate to severe prostration often occurs, with or without loss of consciousness. Death usually occurs within a few hours to a few days although an occasional patient may live even for months. It seems to me that the features against the diagnosis of dissecting aneurysm in this patient are, first of all,



FIG. 1. The gross appearance of the tumor mass in the left adrenal is illustrated. The lesion occupies and replaces the greater part of the organ, only a narrow band of cortical tissue surrounding it at the periphery (light in the photograph). The cut surface of the mass presents a variegated appearance due to an intermixture of areas of necrosis (light) and hemorrhage (dark). The only portion of the adrenal unaffected is shown attached to the tumor below and to the left.

that if you attribute the evidence here for myocardial infarction (if there is such evidence) to an underlying dissecting aneurysm, it would have had to involve the ascending aorta, and the description of the pain was not suggestive of that. Second, how would you account for the guaiac-positive vomitus if the patient had had a dissecting aneurysm? Third, if you say that this patient had a dissecting aneurysm of the aorta to account for her terminal events, there is no very good explanation for the electrocardiographic findings. One would have to say then that she had an independent pulmonary embolus or an independent myocardial infarction. I am assuming, Dr. Schroeder, that the terminal renal events here were just due to shock. Is there any further comment needed on that? The final house staff diagnoses were cholelithiasis; acute cholecystitis, suspected; hypertensive cardiovascular disease; and infarction of myocardium due to arteriosclerotic coronary thrombosis. I personally think this patient had hypertensive cardiovascular disease. I believe she had an infarction of the myocardium due to arteriosclerotic and coronary thrombosis. I am attracted by the possibility that she might have had occlusion of the mesenteric artery due to an embolus from the left ventricle.

PATHOLOGIC DISCUSSION

DR. ROBERT C. AHLVIN: The surface of the body of this well developed, asthenic white woman was not remarkable except for the presence of a well healed, lower abdominal scar. The heart was hypertrophied (470 gm.) and dilated. Coronary arteries were sclerotic and stenosed but not occluded. The myocardium, particularly that of the right ventricle, was diffusely infiltrated with fat, but infarcts were not demonstrable. The lungs were heavy, bright red and crepitant; thrombi were not encountered. Uterus, tubes and ovaries had been removed surgically. The liver was slightly congested. The gallbladder contained two rather large soft stones; its wall was normal and the extrahepatic portion of the biliary tree was widely patent. The surfaces of the kidneys were finely granular, but the organs were otherwise normal.

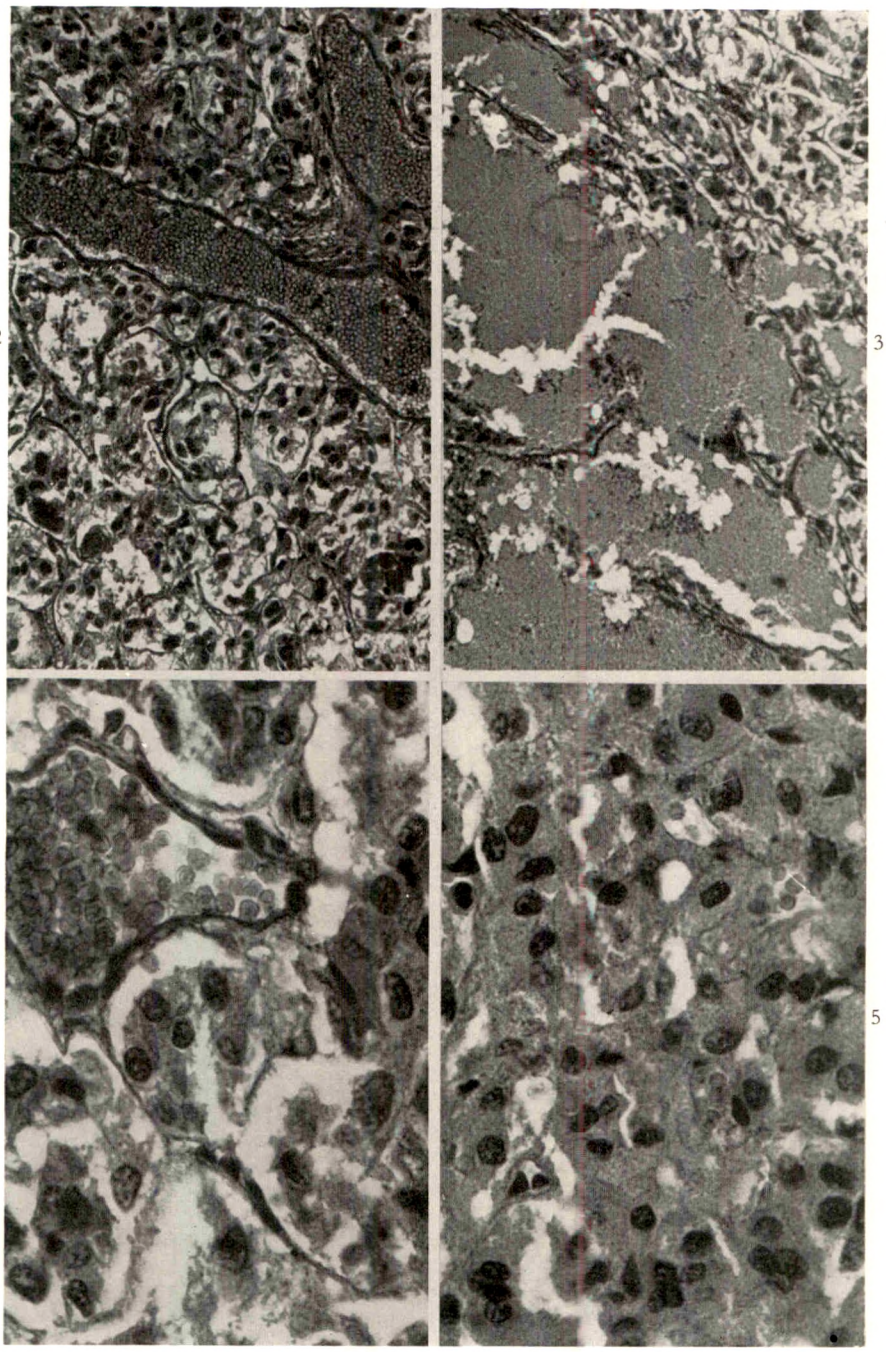
The right adrenal was normal, but the left weighed 50 gm., and had been partially replaced by an orange-yellow mass 4 cm. in diameter (Fig. 1) containing soft, hemorrhagic areas. Bright yellow cortical tissue surrounded the nodule.

DR. W. STANLEY HARTROFT: Microscopically, the tumor in the right adrenal presents a uniform appearance (Fig. 2) interrupted by dilated vessels and hemorrhage. (Fig. 3.) Remnants of cortex at the periphery contain lipid. The cells are arranged in pseudoacini, supported by well defined basement membranes. (Fig. 4.) Nuclei possess prominent nuclear membranes, nucleoli and chromatin; the abundant cytoplasm contains granules. They are blackened by osmic acid and give a positive chromaffin reaction. In many respects the tumor resembles normal adrenal medulla (Fig. 5) and is most certainly a secreting pheochromocytoma. The cortex of the opposite adrenal (left) contains a small adenoma and the organ weighed 10 gm., indicating that it had probably undergone compensatory hyperplasia.

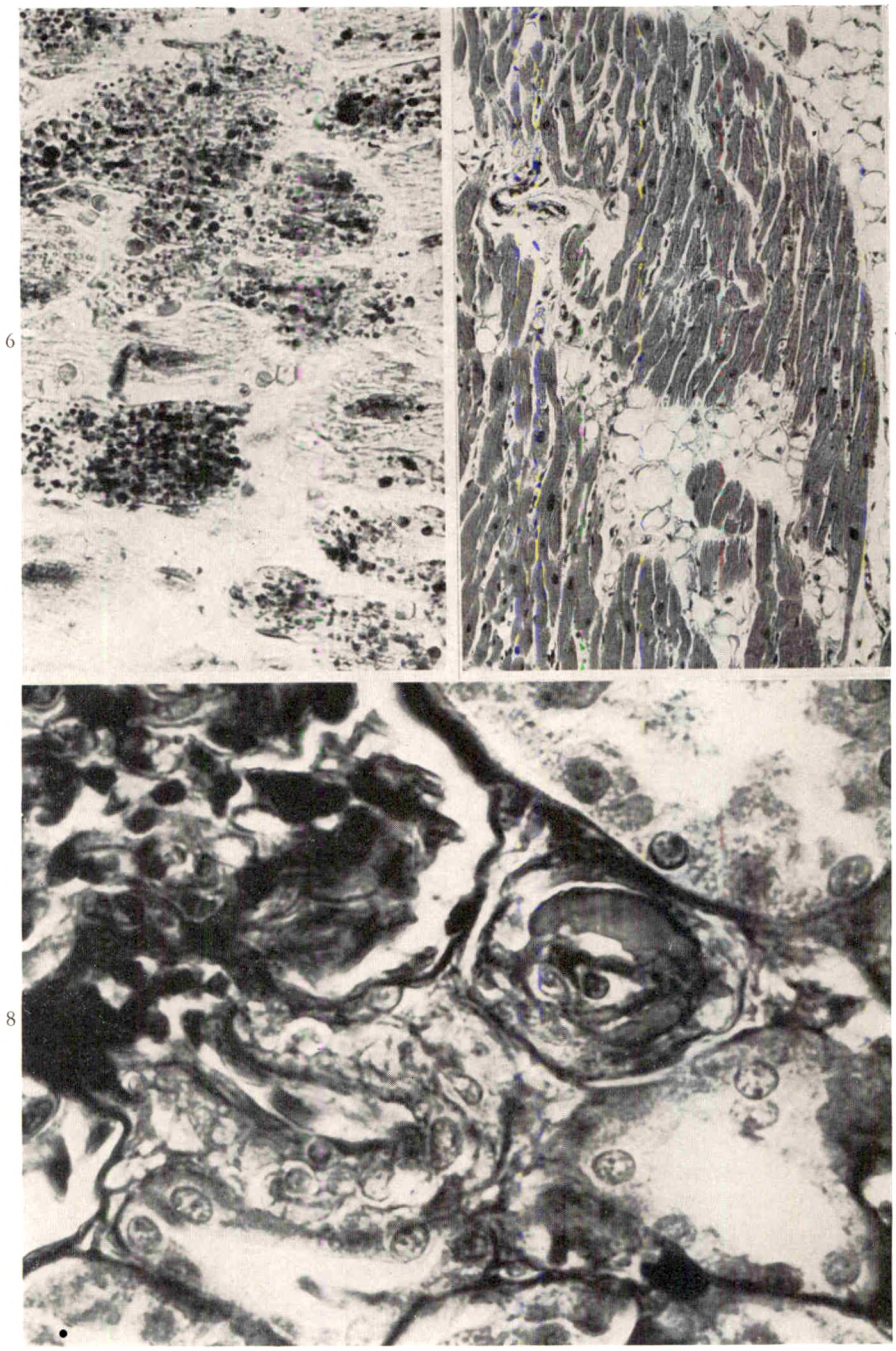
In frozen sections of the heart (left ventricle) stained for lipid, fatty degeneration of the myocardium was appreciable. (Fig. 6.) Fatty infiltration, on the other hand, was unusually prominent in sections of the right ventricle (Fig. 7), although the patient was far from obese (110 pounds). In cases of pheochromocytoma, myocardial degeneration is not infrequently present and may even progress to necrosis.

Renal parenchyma was relatively normal but for minimal degrees of cortical scarring of a nonspecific nature. However, many arterioles exhibited hyperplasia and even necrosis. (Fig. 8.)

Basophils in the anterior pituitary are



FIGS. 2 to 5. These photomicrographs illustrate the appearance of the pheochromocytoma and afford a comparison with the medulla of the contralateral normal adrenal (right). All photographs are of paraffin sections stained with hematoxylin and eosin, photographed through Wratten B and G filters. Figure 2 shows the appearance of one of the better preserved regions of the tumor (original magnification × 200). The acinar arrangement of the cells and large vessels is shown. Figure 3 illustrates another portion of the tumor at the same magnification. Most of the field is taken up by a large area of hemorrhage. A small portion of necrotic tumor tissue occupies the upper right hand corner. Figure 4 illustrates the cytology of the tumor at higher magnification (original magnification × 500). The cells are arranged in acinar forms supported by basement membranes. Note the relation of the tumor cells to the vessel in the upper left. Figure 5 illustrates the appearance of the contralateral, normal adrenal at the same magnification as in Figure 4. The tumor cells closely resemble those of the normal medulla.



FIGS. 6. to 8. These photomicrographs illustrate changes in the cardiovascular system which are probably the result of the hormonal imbalances produced by the secreting tumor cells. Figure 6 is a photomicrograph of a frozen section of left ventricle stained with Oil Red O and Light Green (Wilson). The myocardial fibers are swollen, degenerating and filled with innumerable droplets of stainable fat (black in picture) (original magnification × 400). Figure 7 illustrates fatty infiltration present in the auricles of the heart. There has been extensive replacement of destroyed myocardium by infiltrating adipose tissue Wratten B and G filters; hematoxylin and eosin stain (original magnification × 200). Figure 8 illustrates the appearance of the renal arterioles. To the right of the glomerulus (upper left) is an afferent arteriole cut in cross section. Recent necrosis is present in its media. Below to the left is another portion of the same vessel at the point where it enters the glomerular root. The hyperplastic vacuolated cells probably represent an altered juxtaglomerular unit. The macula densa does not appear in the plane of this section. Masson stain; Wratten B and G filters (original magnification × 500).

vacuolated, enlarged and increased in number. Beta cells of pancreatic islets are normal and their degree of granulation is within normal limits.

This patient suffered from the effects of an adrenal tumor which fulfills all the criteria for the diagnosis of a functional pheochromocytoma. The rest of her organs were unremarkable except that the consequent elevation in blood pressure is reflected by arteriolar hyperplasia with necrosis in the kidney and by cardiac damage (fatty degeneration and fatty infiltration of the myocardium). Vacuolization of basophils is a prominent feature of the pituitary sections and probably resulted from the hormonal imbalance produced by the tumor.

Acknowledgment: Illustrations were made by the Department of Illustrations, Washington University School of Medicine.

Case Reports

Anoxemia Secondary to Polycythemia and Polycythemia Secondary to Anoxemia*

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OUR pulmonary function laboratory is often asked to help in determining whether polycythemia is primary in nature or secondary to anoxemia caused by pulmonary disease. Originally we believed that this differential diagnosis could be made solely on the basis of careful measurements of arterial O_2 saturation. This belief was based both on theoretical and practical considerations. Theoretically, uncomplicated polycythemia vera should not lead to arterial anoxemia because diffusion across the normal alveolar capillary membranes permits the transfer of enough O_2 to saturate fully even twice the usual number of red blood cells per unit of blood per unit time. Furthermore, simple pulmonary vascular congestion, which is believed to accompany the plethoric state in polycythemia, should not lead to any decrease in the diffusing capacity of the lungs. Practically, it has been shown that in the great majority of patients with severe polycythemia the arterial O_2 saturation is within normal limits.[1] We have confirmed this in our studies. We considered, therefore, the normal arterial O_2 saturation at rest and during exercise in a patient with polycythemia as strongly suggesting that polycythemia is primary.

It seemed logical to conclude also that reduced arterial O_2 saturation in a patient with polycythemia indicates polycythemia is secondary to anoxemia. However, this conclusion is questionable on several grounds: (1) Patients with polycythemia vera are usually more than fifty years of age. Healthy persons in this age group may have a slight reduction in arterial O_2 saturation.[2] Further, polycythemia vera may coexist in patients with slight to moderate anoxemia caused by pulmonary disease. The problem in these patients is to determine whether the degree of anoxemia present is likely to have caused the degree of polycythemia. (2) Patients with polycythemia vera occasionally have arterial anoxemia which is considered to be secondary to polycythemia itself.[3–5]

This paper contains a report of a patient with polycythemia referred to us by another doctor† who wished to know whether the polycythemia in this patient was secondary to anoxemia. Arterial blood studies showed that the patient was indeed anoxemic and this suggested that he had secondary polycythemia. However, more complete studies led to the conclusion that anoxemia was caused by localized damage to the medullary respiratory center, possibly caused by emboli or thromboses.

We are also reporting briefly on twenty-four other patients with polycythemia vera who had little or no arterial anoxemia or pulmonary disorders; we wish to stress that anoxemia secondary to polycythemia vera is uncommon and not, as might be inferred from a recent report,[5] a frequent occurrence.

CASE REPORT

Patient N. M. was a large man (S. A. = 2.1 sq. M), sixty-three years old. His first complaints were in 1937 when he developed frequent hiccoughs, sometimes

† Dr. Rodney Kirk of the York Hospital.

* From the Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. Supported in part by a contract between the office of Naval Research and the University of Pennsylvania.

followed by vomiting and blood spitting. However, he was able to work regularly on a farm until July, 1953 when he had a severe hemorrhage, possibly originating in esophageal varices. He also complained of arthritis and, a few months later, of numerous attacks of numbness and tingling on the right side of his body associated with a failing memory. He had no dyspnea except for a short time in June, 1953. Pertinent findings on physical examination were purplish-red cyanosis of the face, numerous telangiectasia, moderate enlargement of the heart and liver (spleen was not palpable), venous engorgement in the optic fundi and Argyll-Robertson pupils. Serum and cerebrospinal fluid tests for syphilis gave negative results and cerebrospinal fluid pressure was within normal limits. X-ray of the chest showed increased markings throughout the lung fields; these were attributed to mild passive congestion. Studies of the blood performed after the severe hematemesis showed: red cells, 6.0 to 6.4 million per cu. mm.; hemoglobin, 20.0 to 20.6 gm./100 ml.; hematocrit, 67 to 70 per cent; white blood count, 6850 to 14,2000/cu. mm. and platelets, 140,000 to 174,000/cu. mm. The measurements that were made on this patient are presented in Tables I and II.

TABLE I
PULMONARY FUNCTION STUDIES ON PATIENT N. M.

Studies	Normal	Patient N. M.
Lung Volumes:		
Inspiratory capacity (ml.)	3,280	3,490
Expiratory reserve (ml.)	820	610
Vital capacity (ml.)	4,100	4,120
Residual volume (ml.)	1,840	1,930
Total lung capacity (ml.)	5,940	6,030
RV/TLC × 100 (%)	42	32
Functional residual capacity (ml.)	2,660	2,540
Respiratory dead space (ml.)	180–200	270
Distribution of Inspired Gas:		
Single breath O_2 test (% N_2)	2.0	1.3
Pulmonary N_2 emptying rate, 7′ test (% N_2)	2.5	0.3
Diffusion:		
Fractional CO uptake (%)	50	48
Diffusing capacity, single breath (ml. CO/min./mm. Hg)	20–40	30
Mechanics:		
Maximum breathing capacity (L./min.)	107	194
1 second vital capacity (%)	81	79
3 second vital capacity (%)	95	92
Maximum expiration flow rate (L./min.)	500	440
Maximum inspiration flow rate (L./min.)	400	202
Miscellaneous:		
O_2 consumption (ml./min.)	300	370
Hematocrit (%)	46	64.2
Hemoglobin (gm./100 ml.)	15.6	18.6
O_2 capacity (ml. O_2/100 ml.)	20.9	24.9
Venous pressure (cm. H_2O)	4–11	8.0
Circulation time, lung to ear (sec.)	6–8	9
Circulation time, arm to ear (sec.)	12–16	20

TABLE II
BLOOD GAS AND VENTILATORY STUDIES ON PATIENT N. M.

Blood Gas Studies	9/29/53	11/10/53	6/10/54
Arterial O_2 saturation (air), %	82	74	62
Arterial O_2 saturation (hyperventilation, air), %	.....	99	.....
Arterial O_2 saturation (O_2), %	100	100	99.8
Arterial O_2 saturation (exercise), %	.....	58	.....
Arterial O_2 saturation (breath-holding), %	.....	35	.....
Arterial O_2 saturation (7.5% CO_2 in air), %	.....	88	.....
Arterial P_{CO_2}, mm Hg (air)	75	68	62
Arterial pH (air)	7.24*	7.20	7.29
Ventilatory Studies	**9/29/53**	**11/10/53**	**6/10/54**
Respiratory minute volume (L./M^2/min.)	4.8	6.1	3.8
Tidal volume (L./M^2/min.)	0.218	0.200	0.136
Respiratory (breaths/min.)	22	30	28
Alveolar ventilation (L./M^2/min.)	1.7	1.8	0.056

* This patient was not able to compensate for the respiratory acidosis; the reasons for this have not been explored.

In our laboratory we classify pulmonary disorders on the basis of alterations in physiologic processes as follows:[6]

1. Disorders of ventilation
   - A. Hypoventilation
   - B. Uneven distribution of inspired gas (in relation to pulmonary capillary blood flow)
2. Impairment of diffusion of gases across alveolar capillary membranes
3. Disorders of pulmonary circulation
   - A. Uneven distribution of pulmonary capillary blood flow (in relation to alveolar ventilation)
   - B. Venous-arterial shunt
4. Abnormalities in erythrocytes or hemoglobin

It soon became apparent that hypoventilation was a major factor in this patient. The evidence was as follows: (1) The patient had CO_2 retention as well as anoxemia. (2) His resting alveolar ventilation [(tidal volume − respiratory dead space) × frequency] was abnormally low in relation to his O_2 consumption. In part this was due to a moderate enlargement of the anatomic dead space to 270 ml. (as measured by the single breath test using the nitrogen meter[7]) but in

larger measure was due to a reduction in tidal volume. (3) The anoxemia in this patient could be relieved by voluntary hyperventilation with air. When he breathed at different tidal volumes (frequency maintained relatively constant), the arterial O_2 saturation became normal for a man of his age group when the arterial saturation is greatly changed by small changes in ventilation. This is illustrated by Figure 1 in which alveolar ventilation is plotted against the maximal possible saturation which it could produce.

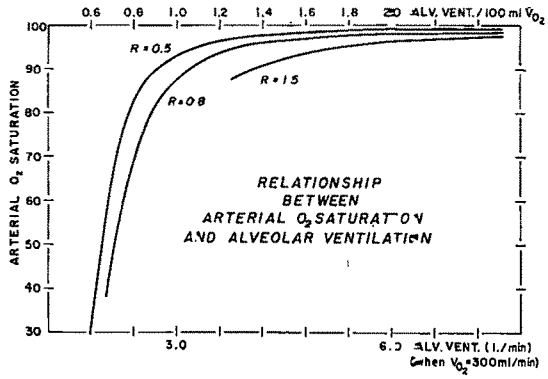


FIG. 1. Relationship between alveolar ventilation and arterial O_2 saturation; these data are derived from the alveolar air equation and the standard oxygen dissociation curve. The data for the bottom scale of alveolar ventilation (V_A) apply when oxygen consumption (V_{O_2}) is 300 ml./minute; those for the upper scale apply to the alveolar ventilation in liters/minute for each 100 ml. O_2 consumption/minute. The values for the arterial O_2 saturation are the maximal possible values at each level of alveolar ventilation because it is assumed that the arterial O_2 tension equals the alveolar O_2 tension. A low respiratory quotient tends to raise the saturation produced by any given alveolar ventilation, but the effect is relatively small. It is meaningless to plot saturations of patient N. M. on this diagram because we do not know the oxygen consumption and respiratory quotient during hypoventilation, nor do we know that the patient was in a steady state. However, the diagram illustrates the theoretic basis for the experimental finding that during hypoventilation arterial saturation is very sensitive to small changes in alveolar ventilation. (Table III.)

alveolar ventilation reached normal values of about 4.0 liters.* (Table III.) When no attempt was made to regulate his breathing, the total ventilation and alveolar ventilation were variable and the arterial O_2 saturation fluctuated widely with the alveolar ventilation.

In normal subjects the sensitivity of the respiratory center to an increase in arterial P_{CO_2} prevents hypoventilation and anoxemia. When arterial O_2 saturation is maintained in the normal range of 95 to 98 per cent large increases in ventilation produce only slight changes in saturation. But when the respiratory center permits hypoventilation, as was the case here, the

* It is of interest that an increase in alveolar ventilation, produced by inhalation of 7.5 per cent CO_2, did not raise the arterial O_2 saturation of this patient to the expected value; this may be due in part to the acidosis produced, with a consequent shift in the O_2 dissociation curve.

Despite this evidence, it seemed important to exclude other pulmonary disorders as contributing to this anoxemia.

TABLE III

EFFECT OF VARIATIONS IN ALVEOLAR VENTILATION UPON ARTERIAL O_2 SATURATION

Respiratory Minute Volume (ml.)	Respiratory Frequency (f)	Tidal Volume (T. V.) (ml.)	Alveolar Ventilation/Breath (TV − DS)* (ml.)	Alveolar Ventilation/Minute (TV − DS) × f (ml.)	% Saturation Arterial Blood (oximeter reading)‡
4,060	22	185	.....†	.....†	55
4,400	22	200	.....†	.....†	65
5,060	24	210	.....†	.....†	80
5,060	24	210	.....†	.....†	65
6,440	24	270	0	0	74
6,530	23	285	15	345	89
6,780	24	285	15	360	84
7,010	25	280	10	250	87
7,240	25	290	20	500	82
8,320	28	295	25	700	86
9,330	26	360	90	2,350	87
9,630	27	355	85	2,300	90
9,680	26	370	100	2,600	89
11,600	29	400	130	3,770	94
12,100	31	390	120	3,720	93
17,450	30	580	310	9,300	95.5
22,400	28	800	530	14,800	97
24,500	29	845	575	16,650	96
24,700	29	850	580	16,800	97
24,150	25	965	695	17,350	96
41,000	28	1,465	1,195	33,500	97
60,400	26	2,320	2,050	53,300	96

* A dead space value of 270 ml. is used in these calculations although it is probably greater at large tidal volumes and less at small tidal volumes.

† When alveolar ventilation is computed in these cases from values for tidal volume and anatomic dead space, negative values are obtained. This occurs because inspired gas does not push the dead space gas ahead of it as a square front but pushes into the dead space as a wedge or cone so that some inspired gas reaches the alveoli before a volume equal to dead space volume has been inspired. Therefore, when tidal volume is so small that it does not completely flush the dead space, the alveolar ventilation/breath is considerably greater than TV − DS.[8] Thus estimates of alveolar ventilation given in the upper half of this table are underestimates.

‡ The arterial O_2 saturations recorded in the table are all "oximeter" saturations. The patient was not necessarily in a steady state since the different rates of ventilations were maintained for only two or three minutes in some cases.

Uneven distribution of inspired gas to the alveoli was excluded by the single breath test utilizing the nitrogen meter[9] and by the pulmonary nitrogen emptying rate test. Furthermore, the lung volumes, RV/TLC ratio, maximum breathing capacity and maximal inspiratory and expiratory flow rates were all within normal limits.

Impairment of diffusion was ruled out by two tests of CO uptake.[10,11] Furthermore, the arterial P_{CO_2} of 75 mm. Hg was incompatible with uncomplicated alveolar-capillary block.

It is possible that multiple pulmonary emboli, thromboses or sludges might produce anoxemia owing to uneven distribution of pulmonary capillary blood flow, even though distribution of inspired gas to the alveoli was uniform. However, calculations of dead space based on Bohr's equation and measurements of arterial and expired P_{CO_2} showed that the dead space volume so computed (240 ml.) was approximately the same as the anatomic dead space (270 ml.). This indicates that the ventilation/blood flow ratios of this patient were not widely different in different parts of the lungs; since ventilation was uniform by the single breath test it is reasonable to assume that the pulmonary capillary blood flow was also fairly uniform throughout the lungs.

Venous arterial shunts were excluded as an important factor by the observation that the blood became fully saturated during hyperventilation with air or during the inhalation of 100 per cent O_2. To eliminate completely the possibility that a small shunt is present, not only should the hemoglobin of arterial blood become fully saturated with O_2 during the inhalation of pure O_2, but an additional 2.0 ml. O_2/100 ml. blood should be present as dissolved O_2. This latter value was never quite attained in our studies on patient N. M. so that a small shunt was not ruled out; however, it could not possibly explain the degree of unsaturation in this case.

Abnormal hemoglobin could not be detected by spectrophotometric studies.* The oxyhemoglobin dissociation curve was normal, considering the arterial pH, and studies of the rate of uptake of CO by the red blood cells showed this to be within normal limits.

These studies confirmed our impression that hypoventilation was the patient's major difficulty. Our next step was to determine the reason for the hypoventilation. The fact that the patient had decreased alveolar ventilation despite the coexistence of three potent stimuli to respiration (anoxemia, acidosis and increased arterial P_{CO_2}) indicated that either there was a serious mechanical hindrance to breathing or that the neuromuscular mechanisms responsible for breathing were depressed. Clearly, since the patient had a maximum breathing capacity of 190 L./minute, there was neither mechanical limitation to breathing nor depression of his peripheral neuromuscular mechanisms, and the lesion, by exclusion, must be in the central respiratory mechanism. This was confirmed by several tests.

1. The patient had an unusually sluggish respiratory response to inhalation of CO_2 on four occasions. The respiratory minute volume increased to only 14 to 21 L./minute even after breathing 7.5 per cent CO_2 for twenty minutes. Dripps and Comroe[12] found that healthy young men responded to the inhalation of 7.6 per cent CO_2 by increasing their minute volume to 52 L./minute (standard deviation ± 15.4). There was considerable individual variation in the response of this group but the response of our patient was less than the mean minus 2 standard deviation. Morton[13] found that the average minute volume during inhalation of 7.2 per cent CO_2 in air was 50 L./minute (standard deviation ± 16.3) and that practically every subject developed dyspnea and headache during inhalation; our patient had no complaints during inhalation of 7.5 per cent CO_2 for more than twenty minutes.

2. Starr and associates[14] found that adrenalin® increases respiratory minute volume; this is believed to be due to direct stimulation of the respiratory center. Subcutaneous injection of 0.5 mg. adrenalin produced no change in respiratory minute volume in the patient.

3. Our patient's respiratory response to exercise was also poor, and arterial O_2 saturation fell markedly. (Table II.) This, added to the finding that ventilation was voluntary at a rate of 190 L./minute, suggests that the voluntary hyperventilation did not require participation of the medullary respiratory center that is responsive to CO_2, acidosis or anoxemic reflexes, but that the hyperpnea of muscular exercise did.

4. The patient also had an abnormal response to breath-holding. He was able to hold his breath for ninety seconds and experienced no serious discomfort during this period. The test was discontinued because of the development of extreme cyanosis; arterial blood drawn after eighty seconds of breath holding showed the arterial O_2 saturation to be 35 per cent at that time and the P_{CO_2} 77 mm. Hg. This failure to respond to anoxemia strengthens the belief that the respiratory response to stimuli from the carotid and aortic chemoreceptors was also reduced.

There seemed to be no depression of the medullary vasomotor center because the patient did not have postural hypotension and responded normally to the feet-down tilt and cold pressor tests. No neurophysiologic abnormalities could be detected: the electroencephalogram was normal and his pain threshhold* was within normal limits.

We have studied many patients with severe anoxemia, acidosis and CO_2 retention in whom hyperventilation was not possible either because of mechanical limitation or because of narcosis. This is the first patient we have observed who was ambulatory, highly cooperative and normal with respect to physiologic tests of his lungs, yet who failed to respond to these stimuli. For this reason we believe he had a lesion in the region of the respiratory center. This lesion might be due to (1) thromboses resulting from primary polycythemia or (2) a primary neurologic or cerebral vascular lesion leading to chronic depression of respiration, chronic anoxemia and secondary polycythemia. The chief complaint of hiccoughs, dating from 1937, may be supportive evidence of the existence of a lesion in the region of the respiratory center. However, we have no way of determining the cause of the lesion during the lifetime of the patient.

* Performed by Dr. David Drabkin.

* Tested by Dr. James Hardy.

TABLE IV

PULMONARY FUNCTION STUDIES IN PATIENTS WITH POLYCYTHEMIA VERA

Initials of Patients	Age (yr.)	Height (inches)	Weight (lb.)	BSA M^2	Sex	Red Blood Count (million/cu. mm.)	Hemoglobin (gm./100 ml.)	Hematocrit (%)	Vital Capacity		Residual Volume		Total Lung Capacity		RV/TLC × 100	Maximum Breathing Capacity		Alveolar Gas Distribution (% N_2) ‡	Seven Minute Washout (% N_2) §
									ml.	%†	ml.	%†	ml.	%†		L./M^2/min.	%†		
W. B.	70	57	100	1.35	F	10.3	18.7	73	1,830	97	.....	...	.....	...	..	37.3	89	1.3	2.0
J. McA.	21	65	105	1.49	F	....	20.1	..	2,710	82	.....	...	.....	...	..	55.7	91	1.0	1.0
R. C.	47	63	120	1.54	F	10.0	21.3	74	2,605	91	.....	...	.....	...	..	23.2	46	...	1.5
F. B.*	55	62	118	1.52	F	6.4	15.8	..	2,280	90	.....	...	.....	...	..	39.1	93	...	...
Y. S.*	75	67	106	1.53	F	....	14.4	..	1,945	104	.....	...	.....	...	..	31.3	75	...	...
B. D.	55	64	130	1.61	M	....	20.7	69	2,980	80					..	60.5	138	1.2	2.0
H. B.	45	69	144	1.78	M	7.0	16.3	55	4,155	95	.....	...	.....	...	..	61.3	94	...	...
M. B.	56	63	138	1.64	F	....	18.4	61	3,230	126	.....	...	.....	...	..	31.4	75	1.5	1.3
J. T.	45	67	186	1.95	M	8.0	17.0	51	3,990	94	.....	...	.....	...	..	62.3	100	...	...
H. R.	63	63	137	1.61	F	11.0	17.0	61	2,360	113	1,260	52	3,620	80	35	29.6	70	1.0	0.5
M. K.	65	64	105	1.44	F	6.1	18.7	61	1,790	85	2,410	99	4,200	92	57	32.0	76	1.4	1.6
P. S.	61	65	132	1.62	M	8.0	17.5	72	3,700	100	2,955	123	5,915	97	50	38.4	87	4.0	2.8
E. V.	61	64	146	1.70	F	....	17.8	64	2,300	109	.....	...	.....	...	..	55.7	113	0.6	1.0
W. K.*	36	68	186	1.95	M	....	16.0	52	3,820	89	1,130	86	4,950	88	23	73.4	109	1.0	1.0
P. M.*	30	67	154	1.79	M	6.5	16.0	48	4,460	105	740	69	5,200	98	14	62.7	89	0.9	0.5
M. N.	57	63	127	1.58	F	8.2	22.4	73	2,660	104	1,655	68	4,315	85	38	60.5	144	1.0	1.4
L. W.*	56	62	180	1.81	M	....	16.1	56	3,650	101	.....	...	.....	...	..	66.9	152	...	3.0
H. W.	59	71	205	2.11	M	....	17.7	56	2,260	60	.....	...	.....	...	..	62.5	142	1.5	...
G. H.*	53	61	161	1.70	F	....	13.4	44	2,480	100	2,395	99	4,875	99	49	38.3	94	1.0	2.5
D. S.	62	65	133	1.65	F	....	18.7	61	2,750	128	915	38	3,665	80	25	46.5	111	1.4	1.5
C. R.	50	63	158	1.78	F	....	18.7	69	3,320	87	2,074	85	5,394	86	38	73.0	174	0.5	0.2
A. L.	69	70	136	1.75	M	7.6	18.6	63	3,870	104	2,462	101	6,332	102	39	37.0	84	2.4	0.9
C. B.	44	75	226	2.30	M	4.5	18.0	51	5,200	109	1,357	93	6,557	105	21	54.5	65	1.3	3.0
F. W.*	47	71	198	2.08	M	....	16.2	45	.....	...	.....	...	.....	...	..	....	...	...	...

* Blood studies performed after therapy for polycythemia.

† % equals per cent of predicted value based on physical characteristics and age.

‡ Normal values for alveolar gas distribution are less 1.5 per cent N_2.

§ Normal values for seven minute washout are less than 2.5 per cent N_2.

We have followed up the patient for more than nine months. During this time therapy has included bleeding, mechanical hyperventilation continuously for as long as four days, O_2 therapy, diamox® and the use of sympathomimetic drugs which have a stimulant effect on the central nervous system. None of these has increased the resting ventilation or respiratory response to CO_2. Arterial saturation was lower during the last study and we suspect that the depression of the central nervous system is not only irreversible but may have become worse because of chronic anoxia and CO_2 retention.

TABLE V

ARTERIAL BLOOD STUDIES IN PATIENTS WITH POLYCYTHEMIA VERA

Initials of Patients	Arterial Blood Studies						
	O_2 Content (vols. %) Rest	O_2 Capacity (vol. %) Rest	O_2 Saturation (%)			P_{CO_2} (mm. Hg) Rest	pH Rest
			Rest	Exercise	100% O_2*		
W. B.	24.9	25.0	99.6	....	.....	37.0	7.39
J. McA.	26.3	26.9	97.5	....	.....	34.5	7.42
R. C.	27.4	28.5	96.7	....	.....	38.5	7.40
F. B.	20.7	21.2	97.4	....	.....	40.8	7.36
Y. S.	17.8	19.3	92.3	....	.....	43.0	7.37
B. D.	26.8	27.7	95.8	....	.....	41.0	7.35
H. B.	21.1	21.8	96.7	....	.....	40.0	7.37
M. B.	23.0	24.7	94.0	....	.....	36.0	7.48
J. T.	21.9	22.8	96.1	....	.....	43.0	7.35
H. R.	21.5	22.8	94.5	....	.....	41.8	7.37
M. K.	23.5	25.1	93.7	....	$100^{+1.5}$	42.0	7.39
P. S.	22.1	23.4	94.7	93.4	$100^{+1.79}$	46.9	7.34
E. V.	23.5	23.8	98.5	....	$100^{+1.81}$	28.0	7.45
W. K.	20.8	21.4	97.0	....	.....	46.0	7.37
P. M.	20.7	21.4	96.6	96.2	$100^{+1.39}$	39.0	7.38
M. N.	29.1	30.0	97.3	95.9	$100^{+2.3}$	35.5	7.37
L. W.	21.2	21.6	98.2	....	.....	36.3	7.45
H. W.	24.2	23.7	95.8	96.2	.....	....	....
G. H.	17.8	17.9	99.0	98.4	$100^{+1.67}$	38.5	7.40
D. S.	23.8	25.0	95.0	95.5	.....	41.0	7.37
C. R.	24.1	25.0	96.0	97.5	$100^{+1.53}$	37.0	7.39
A. L.	24.1	24.9	96.0	92.0	$100^{+2.56}$	37.0	7.37
C. B.	23.2	24.1	96.0	....	$100^{+1.33}$	35.0	7.42
F. W.	19.9	21.7	92.0	98.0	.....	42.0	7.38

* Superscript refers to ml. O_2/100 ml. blood in excess of that required to saturate hemoglobin (i.e., dissolved O_2).

PULMONARY FUNCTION IN PATIENTS WITH POLYCYTHEMIA VERA

Over a period of several years we have studied twenty-four patients who satisfied the criteria of the hematology section for diagnosis of polycythemia vera. (Table IV.†) Arterial O_2 saturation and pulmonary function studies gave normal results in all of these except for one in which the subject appeared to have some coexisting emphysema; however, the arterial O_2 saturation was normal for a sixty year old man, both during rest and exercise. The patient was considered to have had polycythemia vera. (Table V.)

In this group of twenty-four patients vital capacity was 80 per cent or more of the predicted value in twenty-three, maximum breathing capacity was 80 per cent or more of predicted value in seventeen and arterial P_{CO_2} was 43 mm. Hg or less in all but two. The ratio RV/TLC was measured in eleven patients and was normal for the age group in all but one. Distribution of inspired gas was measured in nineteen and was normal in all. Arterial O_2 saturation was measured during exercise in nine; it decreased below the resting value in only one. The uptake

† On request more complete data can be obtained from the American Institute of Documentation.

of carbon monoxide (a test of diffusion across the alveolar capillary membranes) was normal in all four patients tested.

These studies are included to show that serious derangement of pulmonary function is the exception rather than the rule in polycythemia vera. A gross abnormality was found in only one (N. M.) of the twenty-five patients studied, and we cannot yet determine whether the pulmonary disorder was primary or secondary.

Possible disturbances in pulmonary function which may be caused by polycythemia vera are:

1. *Central effects:* Depression of the respiratory center owing to sludging or thrombosis can lead to CO_2 retention, anoxia and further central depression.

2. *Effects on the lungs:* An overload of the oxygenating mechanism has been postulated but probably does not occur because total O_2 transfer per minute is not increased in this disease.

A. *Decrease in diffusing capacity:* Emboli or thrombosis may decrease the pulmonary capillary bed and so decrease the diffusing capacity of the lungs. Vascular congestion may decrease the vital capacity and maximum breathing capacity, but should not impair diffusion.

B. *Variations in the ventilation/blood flow ratios throughout the lungs:* It has been suggested that increase in blood volume may open additional capillaries, increase blood flow in these areas and so distort the normal relationship between alveolar ventilation and pulmonary capillary blood flow.[5] Embolization of part of the pulmonary vascular bed would also lead to alterations in these ratios in different parts of the lungs.

3. *Effects on the thorax:* Sluggish blood flow, caused by increased blood viscosity might lead to easy fatigue on the part of the respiratory muscles and a reduction in maximum breathing capacity. However, many patients with severe polycythemia have a normal maximum breathing capacity. The maximum breathing capacity will certainly be reduced in semicomatose patients or patients sick from other causes.

It appears important, despite the rarity of pulmonary and respiratory derangements in polycythemia vera, to measure pulmonary function more frequently in such patients, with particular emphasis on the reactivity of the respiratory center. Patient 4 of Newman et al.[5] was obviously somnolent, lethargic and apathetic and patient 5 of the same series had an incapacitating degree of lethargy. In such cases central depression is readily recognizable. In our patient there was no indication of central depression except for the inability of the respiratory center to respond to certain characteristic stimuli. Our studies also indicate the value of making estimates of alveolar ventilation as well as measuring the minute volume of breathing.

TABLE VI

HEMOGLOBIN AND HEMATOCRIT VALUES IN PATIENTS WITH CHRONIC ANOXEMIA CAUSED BY PULMONARY DISEASE

Number of Patients	% Arterial O_2 Saturation		Hemoglobin (gm./100 ml.)		Hematocrit (% cells)	
	Mean	Range	Mean	Range	Mean	Range
5	46.2	26.8–59.0	13.8	9.2–16.4	47.6	32.5–56.0
9	75.7	70.2–79.9	13.9	9.8–18.8	48.6	37.6–63.0
29	86.6	80 –89.9	14.2	9.2–16.8	46.8	35.0–57.0
71	92.7	90.2–95.3	13.6	9.8–17.1	43.5	32.0–54.5

DEGREE OF SECONDARY POLYCYTHEMIA IN PATIENTS WITH ANOXEMIA

Because chronic pulmonary disease with anoxemia and secondary polycythemia is found frequently in the same age group as is polycythemia vera, we believe it may be helpful to record here our experience with patients with secondary polycythemia to indicate the degree of polycythemia associated with chronic pulmonary disease. Our data are included in Table VI. We have excluded patients with right to left shunts associated with congenital heart disease or pulmonary hemangiomas; in these, the red blood cell count, hemoglobin and hematocrit values have been uniformly high unless a hemorrhage has occurred. In our patients with chronic pulmonary disease the hemoglobin values rarely exceed 16.5 gm./100 cc. and the hematocrit usually does not exceed 55 per cent. Factors which limit the polycythemic response in patients with anoxemia caused by chronic pulmonary disease may be (1) increased arterial P_{CO_2} values, which occur in many patients with uneven ventilation in relation to pulmonary capillary blood flow, and (2) chronic pulmonary infection.* On clinical grounds alone,

* Hurtado et al.[15] studying residents of high altitude, have found that the polycythemic response is depressed when anoxemia is severe (arterial O_2 saturation less than 60 per cent). We have not observed a similar effect in patients with severe anoxemia owing to venous-arterial shunts at sea level.

if venous-arterial shunts have been excluded, the diagnosis of polycythemia vera is likely in any patient who has a hematocrit greater than 60 per cent and a hemoglobin which exceeds 17 gm./100 cc.

SUMMARY

Pulmonary function studies on a patient with polycythemia, arterial anoxemia and CO_2 retention showed that lung volumes, distribution of inspired air, distribution of pulmonary capillary blood, alveolar-capillary diffusion and mechanics of breathing were within normal limits. The only respiratory defect was an inability to ventilate involuntarily sufficiently to arterialize the venous blood. By exclusion a diagnosis of specific depression of the medullary respiratory center was made. It is not known whether the postulated central lesion is primary or is secondary to thromboses related to polycythemia.

The respiratory and pulmonary disorders which might be caused by polycythemia are discussed. In our experience these have been infrequent. Data obtained in this laboratory on the degree of secondary polycythemia associated with anoxemia of pulmonary origin are summarized.

Acknowledgments: The authors wish to express their appreciation to Dr. David Jacobus of the Department of Medicine, University of Pennsylvania School of Medicine for his cooperation and assistance and to Dr. James Hardy, Dr. David Drabkin, Elizabeth Carlsen and Phyllis Colacicco for special studies done on this patient.

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Adrenal Cortical Carcinoma Producing Solely Mineralocorticoid Effect*

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THE familiar clinical syndromes produced by adrenal cortical hyperfunction are frequently associated with varying degrees of electrolyte and water imbalance. These abnormalities, consisting of sodium retention, increased potassium excretion, expansion of the blood volume and, frequently, hypertension, have been reproduced in experimental animals and man by the administration of large doses of 11-desoxycorticosterone[1] or with aldosterone.[2,3] The metabolic and virilizing abnormalities typical of the spontaneously occurring syndromes are not produced by the experimental use of the mineralocorticoids.

The production of electrolyte and water defects by adrenal cortical hyperfunction or tumors in the absence of the metabolic or virilizing effects has not been reported. The authors had the opportunity of studying a patient with an adrenal cortical carcinoma producing solely mineralocorticoid effect, presumably due to excessive aldosterone secretion.

METHODS

The patient was maintained on a diet providing 5 gm. sodium chloride daily throughout his hospitalization. Daily collections of twenty-four-hour urine specimens were made and serum sodium, potassium, chlorides and CO_2 analyses were performed daily during each period of changing clinical state or therapy. Analyses made on the urines included sodium, potassium, chloride, creatinine, 17-ketosteroids and total or free corticoids. During periods of relative stability in the clinical status these measurements were performed two to three times each week.

Blood volumes were determined by the radiochromium-tagged red cell method.[4] Excretion of 17-ketosteroid in the urine was measured by the method reported by Drekter et al.[5] The Porter-Silber method[6] was used to measure urinary free corticoids and the method of Norymberski[7] was used to determine total corticoids (17-ketogenic steroids).

CASE REPORT

Patient H. W., a sixty year old Negro man, entered the hospital on November 20, 1953, complaining of inability to walk for the previous eight or nine days. He stated that he had been in good health until approximately eight months prior to entry when he noted the gradual onset of severe thirst, polyuria and polydipsia. His appetite remained good and there was no weight loss. Approximately nine days prior to entry he noted the onset of rapid progression of aching and weakness in all extremities. He became unable to lift his arms above the horizontal position or to lift his feet from the floor when standing. There had never been any previous similar episodes.

On his three previous admissions (for hemorrhoidectomy and anoplasty) dating from May to October 1953, the urine was consistently negative for sugar and acetone, and fasting blood sugars were normal on two occasions. An electrocardiogram taken in July 1953, showed "LVH with persistent high U waves." The patient had been hypertensive on all previous admissions. The blood pressure ranged from 200/135 to 176/110. The specific gravity of his urine never exceeded 1.009. Serum urea nitrogen and creatinine determinations were normal on two occasions. In July 1953, a twenty-four-hour urine specimen measured 3,520 cc. and the creatinine clearance was 81 cc. per minute.

Physical examination revealed a well developed, moderately obese, ruddy-faced, elderly

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FIG. 1. The patient in December, 1953; note the absence of "cushingoid" features.

man (Fig. 1), complaining of severe thirst. Blood pressure was 175/112.

The skin was warm and dry and the mucous membranes were very dry. There was no lymphadenopathy and no venous distention. The eyegrounds showed moderate A-V nicking and generalized arteriolar narrowing. The lungs were clear. The heart was enlarged 3 cm. to the left of the mid-clavicular line in the sixth intercostal space; rhythm was normal and no murmurs were heard. The abdomen was soft, without striae or masses. The liver was 1.5 cm. below the right costal margin on inspiration and was non-tender. The spleen was not felt. The genitalia were normal. Deep muscle reflexes were markedly hypoactive, especially the biceps and quadriceps. Muscle power was markedly decreased without evident wasting. The patient was unable to rise from a sitting position. The sensory system was normal.

Laboratory studies on entry were as follows: white cells, 7,800; differential: neutrophils, segmented 78 per cent; non-segmented 3 per cent; lymphocytes, 17 per cent; monocytes, 2 per cent; hemoglobin, 15.1 gm. per cent; packed cell volume, 42 cc. per cent; and corrected sedimentation rate, 11 mm. per hour. Urinalysis revealed a pH of 6.5; specific gravity, 1.005; albumin, trace; sugar, negative; acetone, negative; and centrifuged sediment, negative. Kahn and Kolmer tests were negative. Blood chemistries were as follows: urea nitrogen, 10.0 mg. per cent; creatinine, 1.3 mg. per cent; fasting blood sugar (Somogyi), 71 mg. per cent; blood sugar two hours postprandial, 89 mg. per cent; albumin, 2.4 gm. per cent; globulin, 1.8 gm. per cent; total cholesterol, 222 mg. per cent; cholesterol esters, 134 mg. per cent; sodium, 150 mEq./L.; potassium, 1.8 mEq./L.; chlorides, 90 mEq./L.; CO_2 53 mEq./L.; serum calcium, 4.55 mEq./L.; phosphorus, 0.74 mM./L.; alkaline phosphatase, 3.8 units (King-Armstrong). Arterial oxygen saturation 87.0 per cent; venous oxygen saturation 79.5 per cent. Fasting eosinophil count, 36 and 12 per cu. mm. on separate occasions. X-ray of the chest revealed only moderate left ventricular enlargement. Skull x-rays were normal. Intravenous

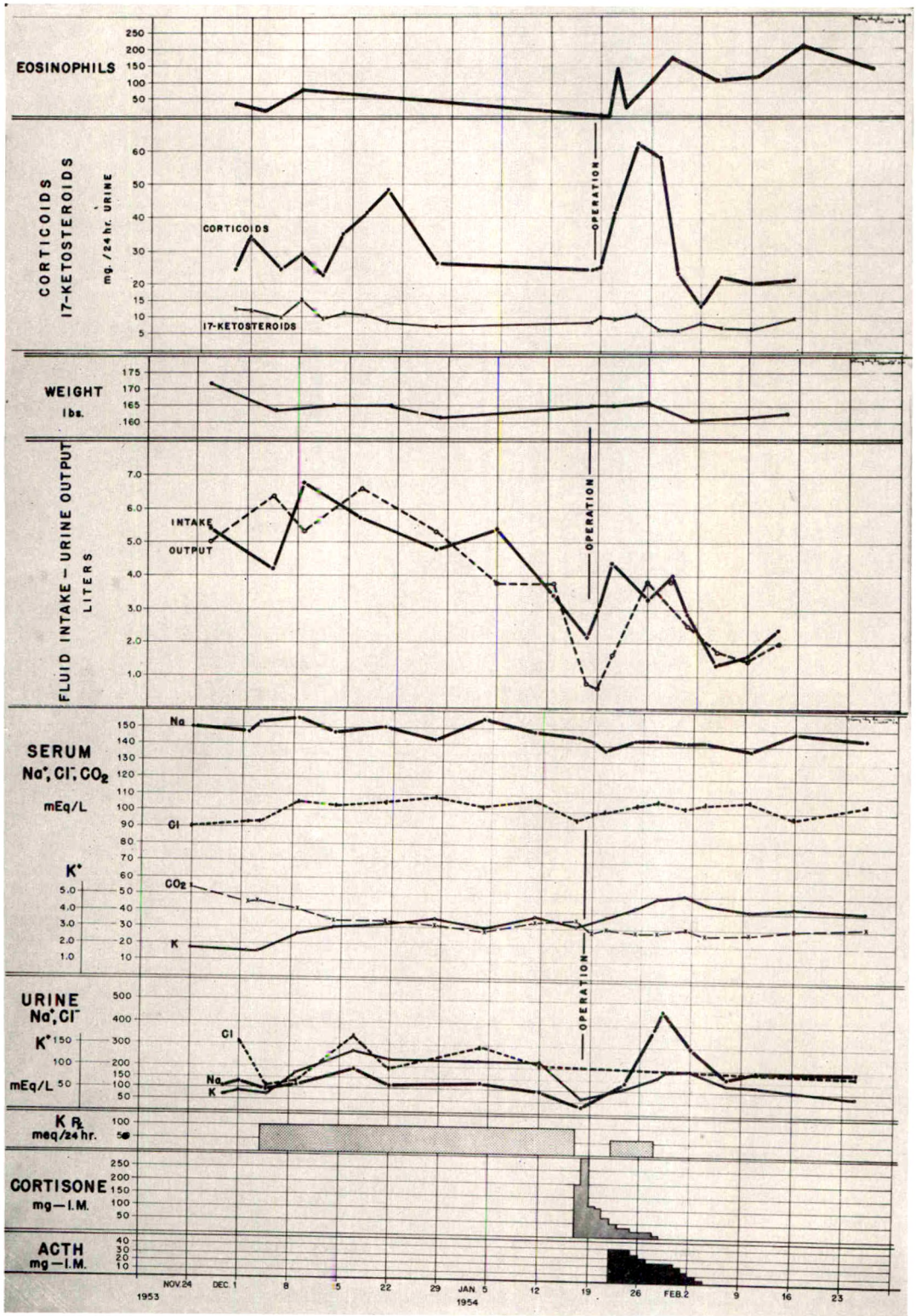
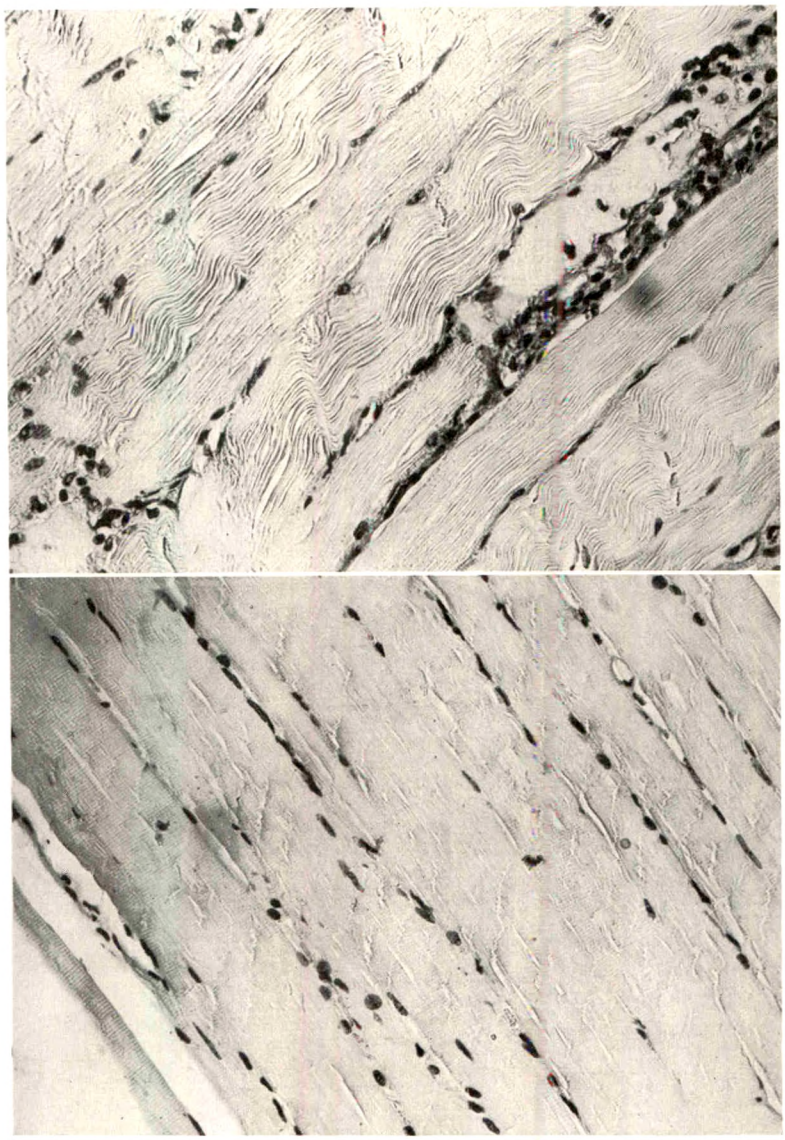


FIG. 2. Summary of observations preceding and immediately following removal of the right adrenal tumor.



3A

3B

FIG. 3. Sections of biceps muscle. A, on entry before potassium replacement; note degenerative changes with some loss of cross-striations and cellular infiltration. B, after potassium replacement showing normal muscle.

pyelograms showed a normally functioning upper urinary tract with no evidence of renal displacement. X-rays did not reveal any evidence of osteoporosis. An electrocardiogram revealed low T waves in V_4 through V_6 with depression of the ST segment and U waves in V_2 through V_5 and was thought to be compatible with left ventricular hypertrophy. Urinary 17-ketosteroid studies were repeatedly normal, ranging from 6 to 15 mg. per twenty-four hours. Urinary corticoid excretions were constantly elevated, the values being 25 to 60 mg. per twenty-four hours (Norymberski method: normal range, 9.6 to 19.2 mg. per twenty-four hours). (Fig. 2.) An electroencephalogram was reported as being mildly, non-focally abnormal. The blood volume was 83 cc. per kg. (normal, 66 cc. per kg.). Biopsy of the biceps muscle revealed swollen fibers showing fatty infiltration and chronic inflammation. (Fig. 3A.)

The fluid intake and urine output ranged between 5 and 7 L. daily and were not influenced by pitressin intravenously or subcutaneously. The urine output responded immediately to restriction of the fluid intake with a resulting severe thirst.

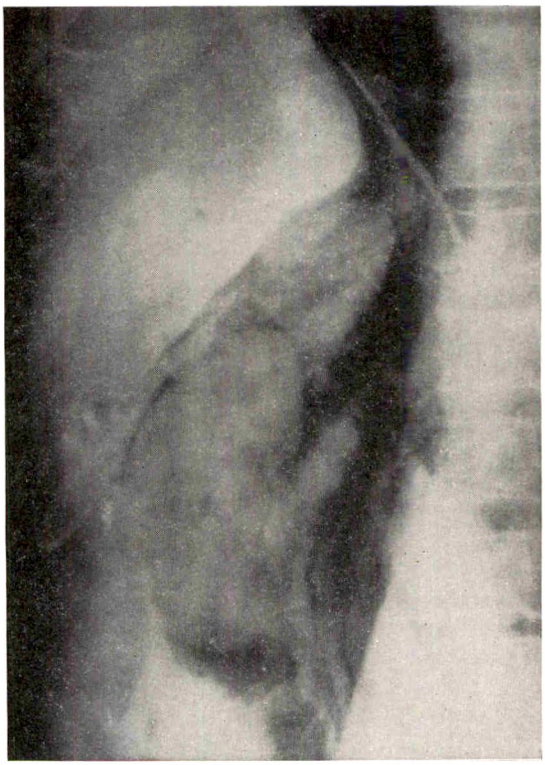


FIG. 4. Retroperitoneal air study showing tumor mass occupying area of right adrenal gland.

On December 3, 1953, the patient was started on a regimen of oral potassium chloride, 100 mEq. daily, with immediate and marked improvement in his thirst, weakness and abnormal reflexes. This therapy had no effect on the fluid intake and output, the patient's weight, the steroid excretion, or the fasting blood sugar. The serum sodium decreased to 145 mEq./L.; the serum potassium increased to 4 mEq./L.; the chlorides increased to 105 mEq./L.; and the CO_2 decreased to 30 mEq./L. The urinary excretion of potassium rose from 30 mEq. per twenty-four hours to approximately 100 mEq. per twenty-four hours. The electrocardiogram showed return of the T wave and ST segment changes to normal and considerable decrease in the size of the U waves. The arterial oxygen saturation increased to 91 per cent and the venous oxygen saturation decreased to 57 per cent.

On January 4, 1954, a retroperitoneal air study showed a right suprarenal, triangular mass which was thought to represent an adrenal tumor. (Fig. 4.)

On January 18, 1954, bilateral adrenal exploration by the lumbar route was performed.

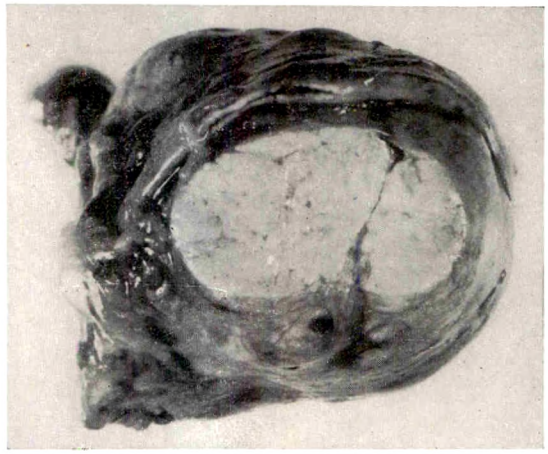


FIG. 5. Adrenal tumor. The cut section was of a canary yellow color.

The left adrenal was found to be normal in size and appearance. However, there was a 4 cm. mass replacing the right adrenal gland which was completely removed. (Fig. 5.) On section this tumor was canary yellow and there was a normal medulla and a small amount of residual cortex tissue. This tumor consisted of three distinct cellular patterns. (Fig. 6.) The patient did very well following surgery and his thirst, strength, fluid intake and output, serum electrolytes, fasting eosinophil count, blood volume, muscle biopsy (Fig. 3B) and electroencephalogram returned to normal. The urine 17-ketosteroids remained normal and the urinary corticoids dropped to a level of 20 mg. per twenty-four hours. The U waves disappeared from the electrocardiogram. The patient was discharged without medications on February 27, 1954.

The patient was readmitted on April 17, 1954, because of return of his thirst, polyuria and polydipsia over the previous ten days. Physical examination revealed a blood pressure of 172/128, pulse 82 per minute, temperature 98.4°F. The liver was 3 cm. below the right costal margin and slightly tender. The neurologic examination was normal. Physical findings were otherwise unchanged since his previous entry. Repeat hemogram, urinalysis, fasting blood sugar and x-rays of the chest and skull were normal. Serum potassium was 2.0 mEq./L.; sodium, 165 mEq./L.; chlorides, 96 mEq./L.; and CO_2, 45 mEq./L. The electrocardiogram at this time showed high U waves and the electroencephalogram again was diffusely abnormal. Excretion of 17-ketosteroid, which was

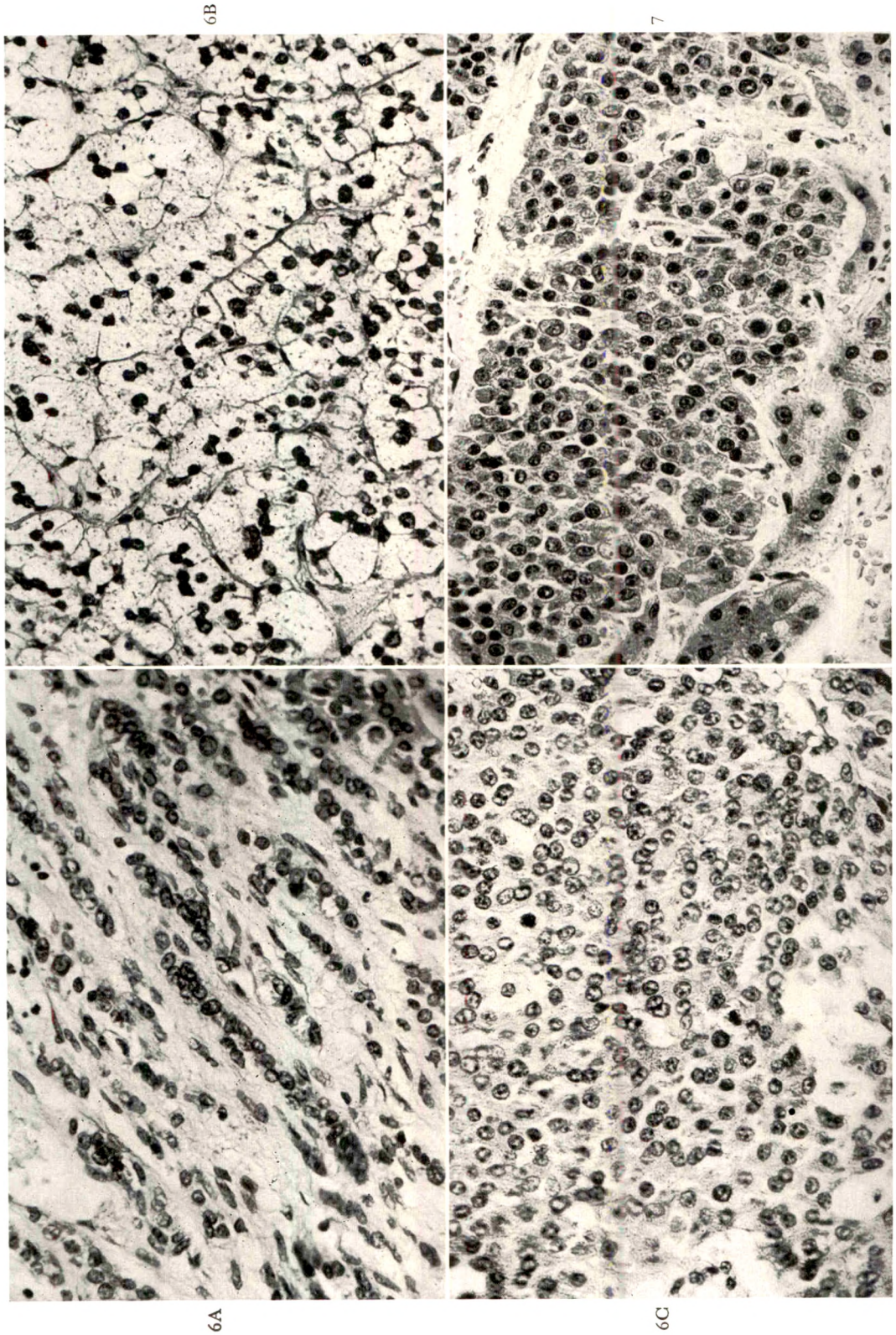


FIG. 6A to C. Sections of the adrenal tumor showing the three predominant cell types which were later found in the metastases examined at autopsy.
FIG. 7. Section of metastasis in the liver (autopsy specimen).

normal initially, rose terminally to the markedly abnormal value of 278 mg. per twenty-four hours and the urinary corticoid excretion rose to 31 mg. per twenty-four hours (Porter-Silber method: normal, less than 1 mg. per twenty-four hours). The fluid intake and output ranged from 2 to 5 L. per twenty-four hours. Fasting eosinophil counts showed a decrease from initially normal values to levels of five to twelve cells per cu. mm. A twenty-four-hour urine specimen was analyzed* for aldosterone content.[8] The resulting value was 12 μg. per 100 minutes (normal 4.8 per 100 minutes), DCA equivalent.

The patient was again started on treatment with potassium chloride supplements, with moderate improvement in his symptomatology and a return to normal of the T and U wave changes present on the electrocardiogram. However, his condition rapidly deteriorated and, on August 15, 1954, masses were felt in the liver for the first time and a x-ray of the chest demonstrated a markedly elevated right diaphragm. Five days later the patient died. Autopsy examination revealed a huge right retroperitoneal tumor mass with numerous metastases to the liver (Fig. 7), lungs and bone marrow. The left adrenal gland was approximately half the normal size. The metastases histologically resembled the cell types present in the original right adrenal tumor.

COMMENTS

This case of adrenal cortical carcinoma is unusual in several respects.

1. The patient on entry had symptoms of severe hypokalemia and clinical findings attributable to an excess of mineralocorticoid activity. At no time did the patient demonstrate the "cushingoid" facies or fat distribution, striae, increased venous pressure, generalized edema or impaired carbohydrate metabolism. To our knowledge a case of adrenal tumor producing an isolated mineralocorticoid excess has not been previously reported.

2. The severity of the hypokalemia was such that the patient was incapacitated by muscular weakness; reflexes were nearly absent; muscle biopsy showed degenerative changes; electrocardiogram showed U waves and ST-T wave changes; electroencephalogram was diffusely abnormal and the arterial oxygen saturation was markedly decreased as was the arteriovenous oxygen difference. All of these findings reverted to normal upon the institution of oral potassium chloride replacement therapy.

The disappearance of the electrocardiographic ST-T abnormalities upon repair of the potassium deficit was quite unexpected since these abnormalities had been attributed to the left ventricular hypertrophy which clinically persisted. However, these electrocardiographic findings reappeared during the patient's relapse and again disappeared promptly when potassium supplements were again administered. (Fig. 8.)

The diffuse electroencephalographic abnormality consisted of the presence of regular, slow 7 to 7½ CPS waves. The electroencephalogram became normal with potassium replacement but upon relapse it again became diffusely although mildly abnormal. (Fig. 9.) This record was characterized by irregular, slow activity which is probably characteristic of a more severe metabolic defect than the original electroencephalogram. These changes have been reported in untreated cases of Addison's disease or in Addison's disease treated only with DOCA.[1]

The increase in the arterial oxygen saturation (from 87 to 91 per cent) and the arteriovenous oxygen difference (from 7.5 to 34.0 per cent) with potassium replacement suggests that the degree of potassium deficit interfered with the ability of hemoglobin to take up oxygen at the lungs and to release it to the tissues. One of us (T. F.) has since produced severe hypokalemia in normal dogs and found a similar decrease in arterial oxygen saturation and A-V oxygen difference which was corrected by potassium replacement.

It is also of interest that during the severe hypokalemia a biceps muscle biopsy revealed muscle cell degeneration and lymphocytic infiltration, whereas after potassium replacement a biopsy from the same muscle was normal.

We postulate that the abnormalities described are due to hypokalemia, which in turn causes intracellular acidosis and thereby interference with oxygen utilization. The resultant tissue hypoxia readily explains all the abnormalities that were corrected by potassium replacement in this patient.

3. Diabetes insipidus was among the diagnoses initially considered in this case. However, the severe electrolyte abnormalities and the failure of the constant polyuria to respond to pitressin, either intravenously or subcutaneously, rapidly eliminated this diagnosis as a possibil-

* Performed by Dr. John Luetscher.

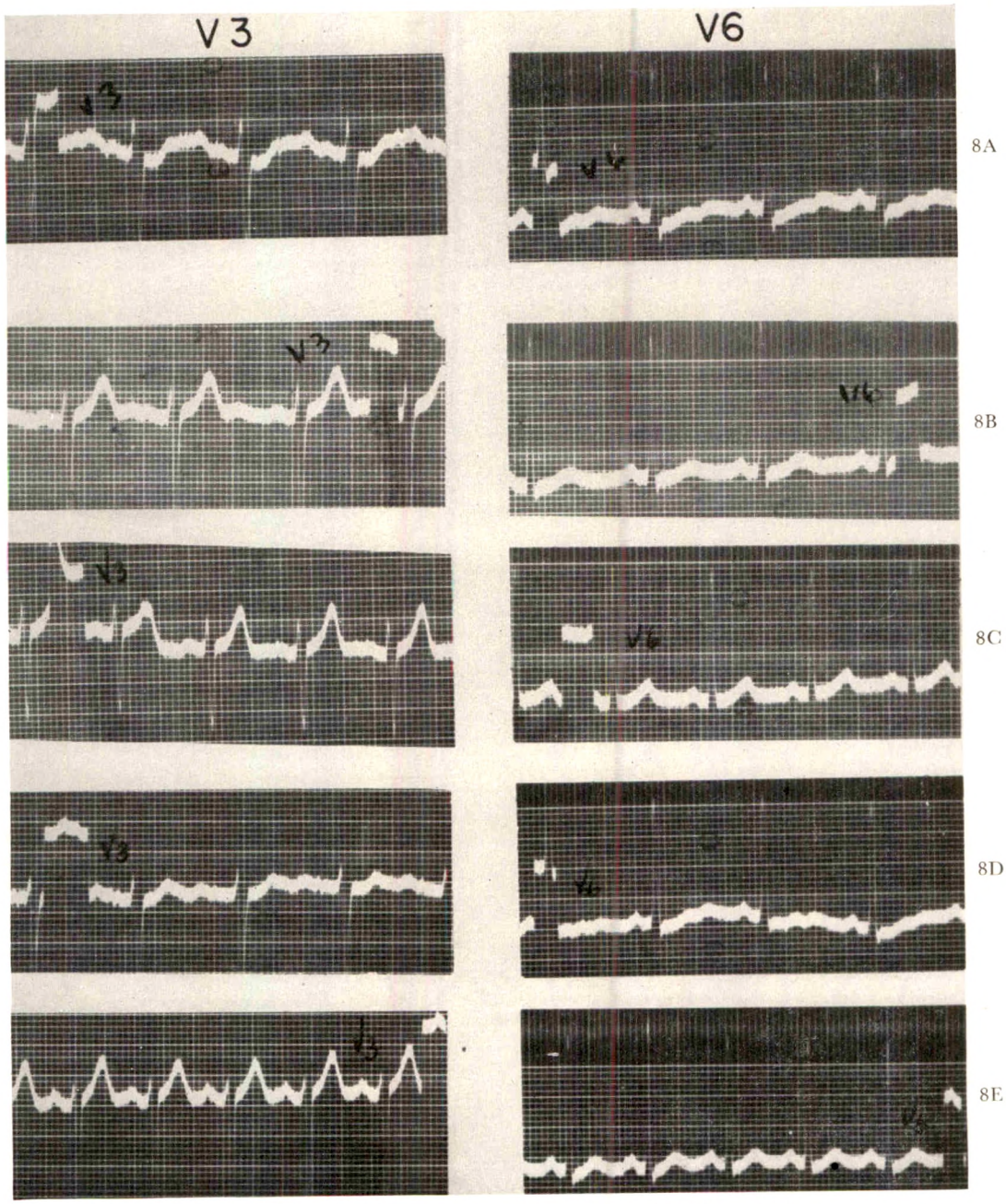


FIG. 8. Leads V3 and V6 showing the alterations in the ST segment, T wave and U wave with varying serum potassium levels. A, November 23, 1953 (on entry): K^+ = 1.3 mEq./L.; CO_2 = 53.0 mEq./L. B, December 18, 1953 (potassium replacement): K^+ = 3.0 mEq./L.; CO_2 = 33.4 mEq./L. C, February 5, 1954 (after tumor removal): K^+ = 4.3 mEq./L.; CO_2 = 28.1 mEq./L. D, April 20, 1954 (relapse): K^+ = 2.3 mEq./L.; CO_2 = 46.4 mEq./L. E, August 6, 1954 (potassium replacement): K^+ = 5.4 mEq./L.; CO_2 = 30.4 mEq./L.

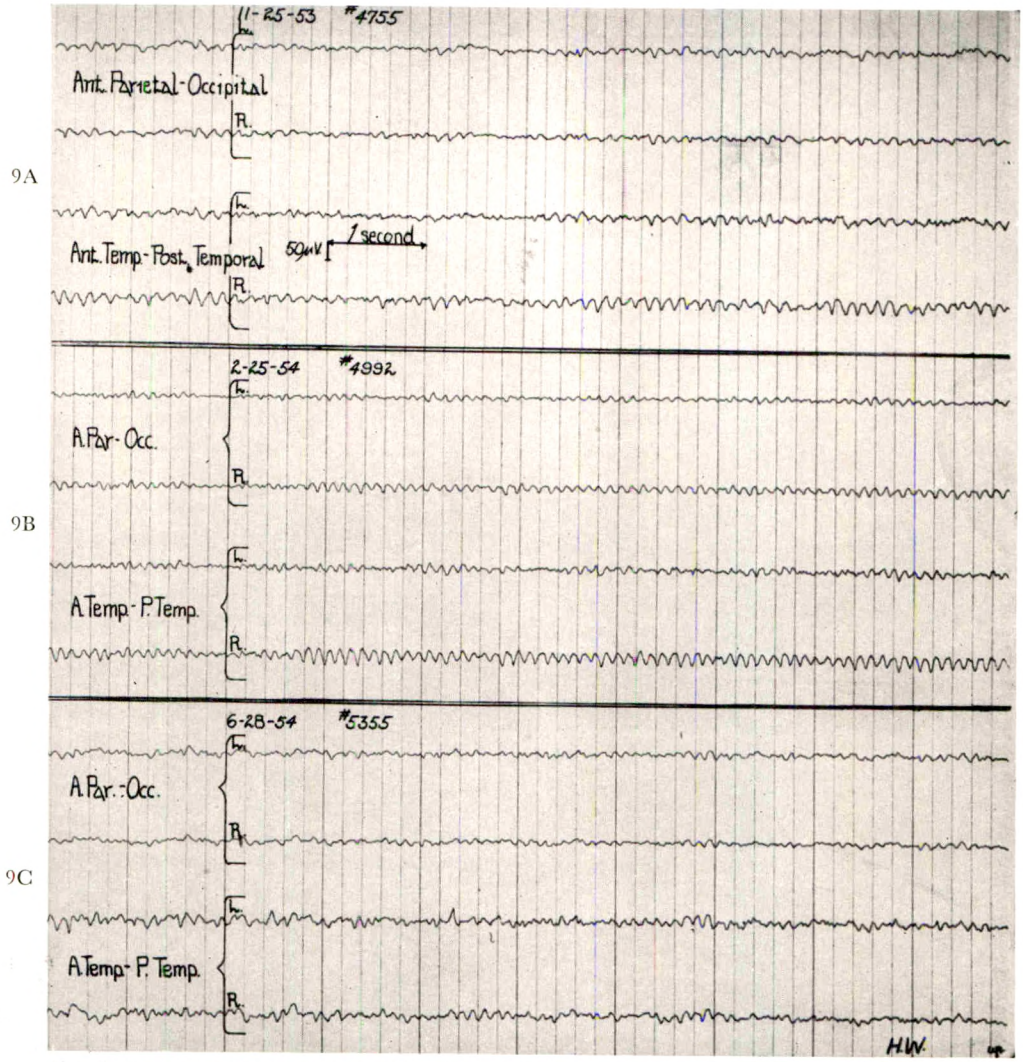


FIG. 9. Representative segments of the electroencephalograms taken at different phases of the patient's illness. The record of June 28, 1954, was made after potassium therapy had been instituted for relapse. The urinary steroid excretion at this time was markedly elevated. A, November 25, 1953 (on entry): K^+ = 1.7 mEq./L.; CO_2 = 46.2 mEq./L.; "mildly, diffusely abnormal due to regular slow, 7–7½ CPS waves." B, February 25, 1954 (after tumor removal): K^+ = 3.7 mEq./L.; CO_2 = 30.9 mEq./L.; "normal EEG." C, June 28, 1954 (relapse): K^+ = 3.9 mEq./L.; CO_2 = 33.3 mEq./L.; "mildly, diffusely abnormal due to irregular, slow, 6–7 CPS waves."

ity. The 5 to 7 L. urine output dropped immediately to below 2,000 cc. when water was withheld, although this resulted in severe thirst. We believed that the polyuria was secondary to the severe thirst and resultant polydipsia caused by the marked hypernatremia. The hypernatremia, thirst and polydipsia improved somewhat on potassium replacement and returned completely to normal upon removal of the adrenal tumor. A "diabetes insipidus-like" syndrome characterized by thirst, polydipsia, polyuria, sodium retention and potassium loss has been produced in normal dogs by the administration of DOCA.[9]

4. Throughout the initial phase of the patient's illness and for three months after his adrenalectomy the urinary 17-ketosteroid excretion remained normal. Then coincident with the onset of his gradual decline the excretion values began to rise, terminally reaching the value of 278 mg. per twenty-four hours. Throughout his course the urinary corticoids

were elevated and returned to normal levels only during the three months following adrenalectomy. Thereafter they rose to markedly abnormal levels. During this latter period urinary aldosterone assays were also elevated. Since aldosterone is not measured in the 17-ketosteroid or corticoid tests, and since there was no evidence in this patient of any effects not attributable to mineralocorticoid activity, we are unable to explain the source of these urinary steroids.

SUMMARY

A case of carcinoma of the adrenal cortex producing solely mineralocorticoid effect is presented and some of the manifestations briefly discussed. The abnormalities produced by the resulting severe hypokalemia seem of special interest and suggest impairment of oxygen transport and utilization, with interference with cerebral, myocardial and muscle metabolism.

Acknowledgments: Many people contributed to the study of this case. Our special thanks to Dr. Forrest M. Willett, Dr. Robert Fischer and Dr. Peter Lewis for their assistance and advice, to Miss Barbara Chapman for the steroid analyses, and to Miss Kay Hyde for the preparation of the charts.

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Spontaneous Hypopotassemia, Hypomagnesemia, Alkalosis and Tetany due to Hypersecretion of Corticosterone-like Mineralocorticoid*

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ABNORMALITIES of electrolyte metabolism in classic forms of Cushing's syndrome, with concomitant disturbances of carbohydrate and protein metabolism, are well known and are explainable by excessive secretion of adrenal cortical hormones.[1–4] No case, however, has been reported in which the disorder has been limited to the electrolyte metabolism. The present study describes a case of spontaneous hypopotassemia, hypomagnesemia, alkalosis and tetany due to hypersecretion of corticosterone-like or aldosterone-like mineral corticoids.†

CASE REPORT

R. G. (27817), a thirty-three year old Negro woman, was admitted to Detroit Receiving Hospital on January 24, 1954, because of painful tetanic contractions of her hands of one day's duration. For approximately two months before admission the patient had noticed muscle weakness, pain and cramps, together with numbness of the extremities, restlessness and nervousness. Six months previously, during the fourth month of pregnancy, she had noticed intermittent spasms of her hands. There was no history of vomiting, diarrhea or ingestion of any medications except occasional salicylate compounds for headaches. She had had mild hypertension for five years and periodic frontal headaches for ten years. Twelve weeks prior to admission mild pre-eclamptic toxemia of pregnancy had developed but the patient delivered a normal fetus three weeks later at another hospital. During the confinement, prior to delivery, the patient was slightly edematous and the blood pressure was elevated to 170/108 mm. mercury. The plasma sodium was 131 mEq./L., potassium 3.35 mEq./L., chloride 94.5 mEq./L., CO_2 combining power 53.2 vol. per cent, serum uric acid 7.0 mg. per cent, and blood non-protein nitrogen 26 mg. per cent. Shortly after delivery, bilateral tubal ligation was performed.

Physical examination revealed a well developed and well nourished patient, acutely ill with obvious tetany. A grade 2 hypertensive retinopathy was present. A positive Chvostek sign was elicited. The heart was slightly enlarged and the blood pressure was 170 mm. Hg systolic and 90 mm. Hg diastolic. No masses or organs were palpable in the abdomen. There was no evidence of Cushing's syndrome or adrenogenital syndrome.

Tetany was readily relieved by rebreathing her own expired air from a paper bag, but the Trousseau sign remained positive. Biochemical studies, summarized in Table 1, showed the presence of hypopotassemic alkalosis with plasma potassium concentration of 1.6, sodium 143, chloride 84, and CO_2 content of 42 mEq./L. The serum calcium was 4.25 mEq./L. by a standard chemical method and 3.95 mEq./L. by the spectrographic method.[5] The serum magnesium was 1.1 mEq./L. and inorganic phosphorus 2.7 mg. per cent. The arterial blood

† Dr. Jerome W. Conn, in his presidential address at the 27th Annual Meeting of the Central Society for Clinical Research, described a case identical with the case presented here, and called it "primary aldosteronism."

* From the Departments of Medicine, City of Detroit Receiving Hospital and Wayne University College of Medicine, Detroit, Michigan. Presented before the 27th Annual Meeting of the Central Society for Clinical Research, Chicago, Illinois, October 29, 1954. Supported in part by grants from the National Institute of Health, the American Heart Association and the Michigan Heart Association.

H was 7.5 and the derived pCO_2 was 56 mm. Hg. The blood urea nitrogen was 13 mg. per cent and the fasting blood sugar on the second day was 76 mg. per cent. Serum albumin was 4.4 and globulin 2.3 gm. per cent.

The initial urinalysis (uncatheterized specimen) show a specific gravity of 1.014, albumin 2 plus, sugar 1 plus, acetone 4 plus, and white blood cells of 15 to 18 per high power field. Catheterized urine on the following day showed a trace of albumin, no sugar, no cellular elements and specific gravity of 1.004. The blood hemoglobin was 12.4 gm. per 100 ml., white blood count 10,500 per cu. mm., with 71 per cent neutrophils, 15 per cent lymphocytes, 9 per cent monocytes and 5 per cent eosinophils. The total eosinophil count was 283 per cu. mm. The phenolsulfonphthalein excretion was 25 per cent in twenty minutes. The electrocardiogram revealed flat to inverted T waves, prominent U waves, and prolonged S-T segment, indicative of hypopotassemia and hypocalcemia. Intravenous pyelographic studies were normal. Presacral air insufflation studies were also normal.

TABLE I

BIOCHEMICAL STUDIES BEFORE AND AFTER TWENTY-ONE DAYS OF INTERMITTENT POTASSIUM CHLORIDE THERAPY*

Blood and Urine Values	Initial	After Treatment
Plasma Na (mEq./L.)	143	150
Plasma K (mEq./L.)	1.6	3.7
Plasma Cl (mEq./L.)	84	101
Plasma CO_2 (mEq./L.)	42	28
Serum Ca (mEq./L.)	4.25	4.75
Serum Mg (mEq./L.)	1.12	.....
Serum P (mg.%)	2.7	2.6
Blood pH	7.5	.....
Blood pCO_2 (mm. Hg)	56	.....
Blood urea nitrogen (mg. %)	13	.....
Urine pH	6.5	.....

* 6 gm./day.

The patient was given 20 ml. of 10 per cent calcium gluconate intravenously without alleviating the positive Trousseau sign. Infusion of 40 mEq. of potassium chloride solution produced no change in the degree of muscle irritability.

After supplementary potassium chloride therapy of 80 mEq. per day, the plasma sodium was 150, potassium 3.7, chloride 101, and CO_2 content 28 mEq./L. Muscle irritability, as judged by the Trousseau sign, gradually disappeared and muscle strength improved markedly.

Further studies were carried out to determine the mechanism of these biochemical abnormalities.

METHODS

Complete metabolic balance studies for Na, K, Cl, Mg and N were conducted for thirty days following readmission on May 6, 1954. Intake was analyzed by a double tray technic, wherein a duplicate tray of food and leftovers of an identical tray served to the patient were analyzed separately. Three-day pooled samples were homogenized and aliquot samples were obtained for analysis. Stools and urines were also pooled and analyzed every three days. Calculation of intracellular balance was made by assuming extracellular confinement of chloride.[6,7] Corrections for nitrogen balance were not made because of its relative stability.

Serial specific renal function studies, using inulin[8] and para-amino hippurate clearances, were made with two to three ten-minute collections of urine.[9] On one occasion endogenous creatinine clearance × 0.9 was used to estimate the rate of glomerular filtration. Tubular reabsorption of water and electrolytes was calculated from clearances of inulin.

Urinary steroid excretion studies* were made on twenty-four-hour collections of urine, treated with ammonium sulfate, acidified to pH 2.0, and extracted four times (one minute each) with n-butanol (or 2:1 ether-ethanol mixture). Blue tetrazolium reducing chromogens were determined by a modification of the method of Chen, Wheeler and Tewell,[10] with incorporation of an internal standard in the analysis and thirty-minute color development. Phenylhydrazine reducing chromogens were determined by a modification of the Porter-Silber reaction,[11] carried out at room temperature with addition of an internal standard control in the procedure.

Bioassays of salt-retaining urinary corticoids were made according to the method described by Johnson, using log K/Na values.[12]†

Sodium and potassium were determined by

* These studies were undertaken by Dr. William Q. Wolfson of the Department of Medicine, Wayne University College of Medicine.

† The bioassay studies were conducted by Dr. Harry W. Hays of the Department of Physiology and Pharmacology, Wayne University College of Medicine.

means of the Beckman flame photometer, according to the method described previously.[13] Chloride was determined by the method of Schales and Schales[14] for plasma and urine, and Whitehorn[15] for stool and food. Serum and urine magnesium was determined by the Titan-

presence or absence of intrinsic organic renal disease, are depicted in Figure 1. Each of these studies was performed after withholding potassium chloride supplements for five to twenty days before the test. Thus at the time of each test there was an acute lowering of the plasma potassium

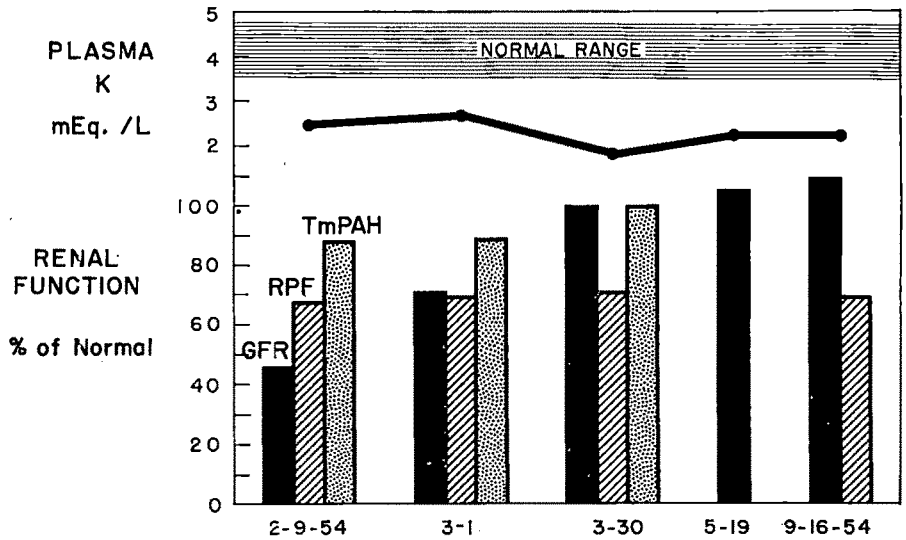


FIG. 1. Serial discrete renal function studies depicted in per cent of normal, showing gradual improvement in glomerular filtration rate and Tm_{PAH} in spite of persistently recurring hypopotassemia. Each study was carried out five to twenty days after temporary cessation of potassium chloride therapy.

yellow method, using starch colloid stabilizer.[16] Stool and food magnesium was determined in a similar manner after digestion with perchloric-nitric acid mixture. Nitrogen was analyzed by the macro-Kjeldahl method.

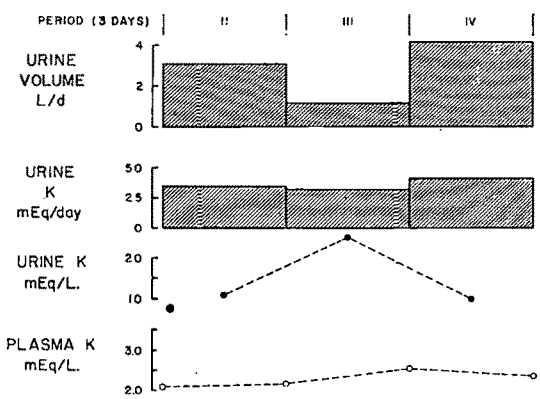


FIG. 2. Relationship of urinary volume to urine potassium excretion.

RESULTS

Renal Function Studies. Serial discrete renal function studies, carried out to determine the to subnormal levels. The initial study on February 9, 1954, revealed a markedly depressed glomerular filtration rate (GFR 55.5 ml./min.), a moderately depressed effective renal plasma flow (RPF 445 ml./min.), and a slightly decreased maximal capacity of the tubules to excrete para-amino hippurate (Tm_{PAH} 63.1 mg./min.). Within three months both GFR and Tm_{PAH} were normal, but RPF remained low at 70 per cent of normal.

Since the patient showed a moderate degree of polyuria, it was necessary to determine whether or not this was a factor in the development of hypopotassemia. Figure 2 illustrates the relationship of urine volume to urine potassium concentration and potassium excretion, determined during a metabolic balance study on a 50 mEq./day potassium diet. Sharp reduction of urine volume from 3.05 L. per day to 1.20 L. per day by restriction of fluid intake resulted in a sharp rise in the urinary potassium concentration from 11.9 to 25.5 mEq./L. The specific gravity of the urine rose from 1.003 to 1.010. With unrestricted fluid intake and a consequent rise in urine volume, the urinary concentration

of potassium again decreased and the specific gravity fell to 1.002. The total quantity of K excreted into the urine varied very little with changes in urinary volume. (Excretion of magnesium was also independent of urinary volume.)

plasma potassium levels and a control twenty-four-hour urine were obtained. (Table II.) The patient was then given 80 mEq. of KCl supplements per day for eight days. Urine collections were obtained daily for the first two days and three-day pooled urines were obtained during

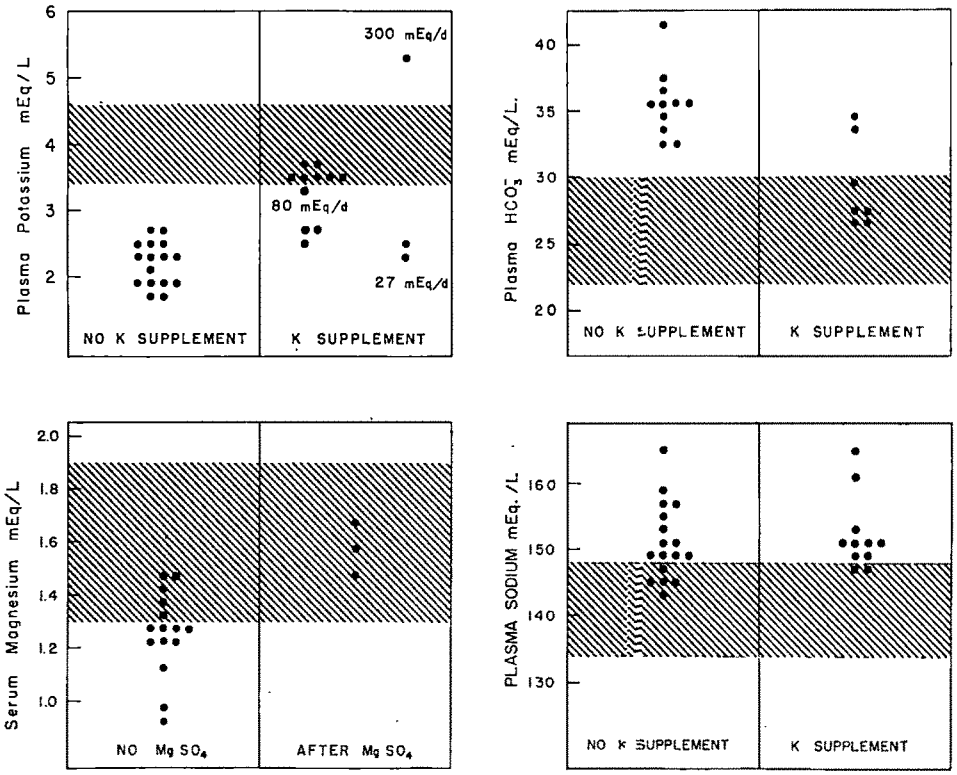


FIG. 3. Plasma potassium, sodium and bicarbonate levels before and afterpotassium supplements, and serum magnesium levels before and after magnesium supplements.

Studies on Potassium Metabolism. During the ten-month period of observation potassium chloride supplements were given intermittently and plasma potassium levels were determined serially. (Figure 3.) When K supplements were withheld, the plasma level fell to values between 1.8 and 2.8 mEq./L. On several occasions a fall to subnormal levels occurred within five days after cessation of therapy. With 80 mEq. of potassium chloride supplements per day, low normal levels were usually obtained, but occasionally during outpatient visits subnormal values were observed. Supplements of 250 mEq. per day for six days raised the plasma potassium level to 5.3 mEq./L. and supplements of 72 mEq. per day had no effect on the plasma level.

Immediately upon initial admission to the hospital, the patient was given a diet calculated to provide 60 mEq. K^+ per day. Two control

the next twelve days. The diet and stools were not analyzed in this phase of the study.

The initial urine contained only 3.34 mEq. of potassium per liter and the urine/plasma potassium concentration ratio was 1.86. On the following day, with institution of potassium supplements, the urinary concentration was still only 6.28 mEq./L., with a U/P ratio of 2.50. Thereafter, a potassium U/P ratio less than 4.1 was never observed.

Estimating approximately 20 mEq. of K in the stool per day, there was a positive balance of some 55 mEq. of K per day during the eight days of KCl therapy and a negative balance of about 15 mEq. per day during the six days without therapy. The plasma potassium concentration rose from 1.8 to 3.7 mEq./L. with therapy and fell again to 2.7 mEq./L. after cessation of treatment.

Sodium diuresis occurred with potassium therapy. This was most marked at the onset of treatment and much less after five days.

A more precise metabolic balance study, including analyses of stool and food, was conducted four months later. (Table III, Fig. 4.)

TABLE II
EFFECT OF SUPPLEMENTARY KCL THERAPY DURING INITIAL ADMISSION ON URINARY ELECTROLYTE EXCRETION

Day	K Intake Supplement* (mEq.)	Urine				Plasma (mEq./L.)			
		Vol. (ml.)	K (mEq.)	Na (mEq.)	Cl (mEq.)	Na	K	Cl	CO_2
1	..	1,360	5	6	6	143	1.6	84	42
2	80	3,230	20	103	117	141	1.8	..	..
3	80	3,290	72	244	244	137	2.5	..	42
4	80	3,617	64	143	147	150	2.5	..	..
5	80	3,617	64	143	147	147	2.6	96	35
6	80	3,617	64	143	147	...	...	..	..
7	80	3,768	72	81	97	...	...	..	..
8	80	3,768	72	81	97	143	3.4	..	34
9	80	3,768	72	81	97	...	...	..	..
10	..	3,547	49	36	57	...	...	..	..
11	..	3,547	49	36	57	...	...	..	..
12	..	3,547	49	36	57	...	3.7	..	..
13	..	3,243	58	42	52	...	...	..	..
14	..	3,243	58	42	52	...	...	..	..
15	..	3,243	58	42	52	...	...	..	..
16	..	....	..	...	...	143	2.7	92	33

* Diet = 60 mEq./day.

Plasma potassium concentration remained subnormal all during the first four periods, each of three days, with an average potassium intake of 50 mEq. per day. Urinary and fecal excretion of potassium was exceedingly large at first, amounting to 98 per cent and 70 per cent of the intake, respectively, but gradually decreased with time. The extremely low stool K during period IV was undoubtedly due to a temporary delay in passage of stool, as indicated by the small quantity passed during this period and the large quantity during the following period.

As to total external exchange of potassium, negative balances of −82 and −18 mEq. were observed during the first two periods, equilibrium being established during the next two periods, even with persistent hypopotassemia. Total sodium balance was markedly positive (+213 mEq.), and chloride balance negative (−141 mEq.) during the first four periods of low potassium intake. Total magnesium balance was significantly negative (−51 mEq.) during the same interval.

Calculation of extracellular and intracellular changes (Table IV, Fig. 5) revealed an early minimal loss of intracellular potassium and

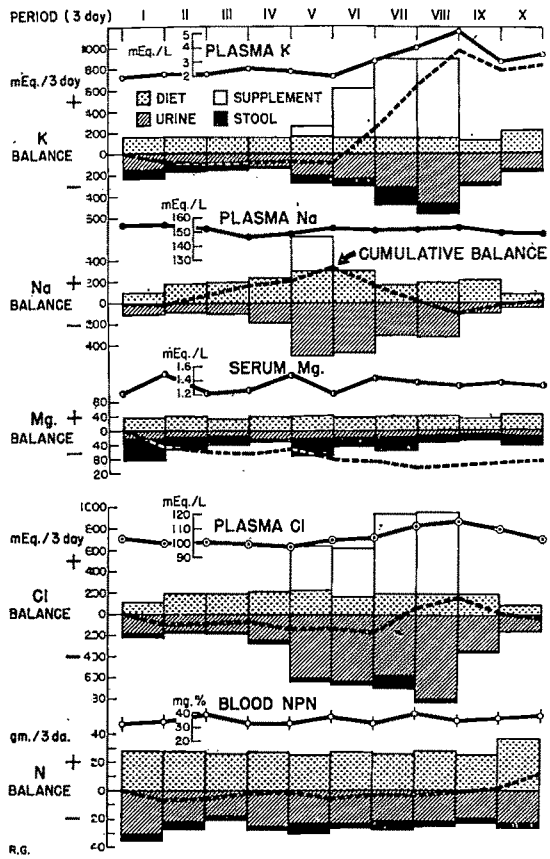


FIG. 4. Complete metabolic balance of K, Na, Mg, Cl, and N during low and high intake of potassium. Stool Na excretion was so small that graphic representation was not possible.

continuous gain of intracellular sodium during the period of low potassium intake. No consistent change in intracellular water content was observed, but a slight decrease in extracellular water was noted. Chloride loss was of the same magnitude as extracellular sodium loss and proportionately greater than extracellular water loss, reflecting the development of extracellular alkalosis.

Results of changes during period V are difficult to analyze because of the administration of parenteral KCl and NaCl.

During periods VI, VII and VIII potassium intake was increased to 200 and 300 mEq. per

TABLE III
METABOLIC DATA

Period (3 days)	Intake*							Urine						Stool						Plasma		Serum					Blood	Body Weight
	H_2O	Food	Na	K	Mg	Cl	N	Vol.	Na	K	Mg	Cl	N	Weight	Na	K	Mg	Cl	N	Na	K	Mg	Cl	CO_2	Ca	P	NPN	
	(ml.)	(gm.)	(mEq.)				(gm.)	(ml.)	(mEq.)				(gm.)	(gm.)	(mEq.)				(gm.)	(mEq./L.)		(mEq./L.)			(mg. %)		(mg. %)	(kg.)
I	7,005	3,290	102	154	40	117	28.6	9,955	112	148	22	188	30.2	636	7	88	64	27	5.6	155	1.9	1.2	104	34	10.2	2.9	32	75.1
II	4,880	4,400	187	156	41	196	28.0	9,160	89	110	19	168	21.2	629	8	64	33	15	5.8	155	2.2	1.5	100	33	....	...	34	74.4
III	1,500	4,251	193	151	35	195	26.5	3,605	98	92	17	182	17.6	456	5	53	24	7	4.1	153	2.2	1.2	101	32	9.3	3.2	39	74.7
IV	10,340	4,410	240	147	41	216	27.7	12,545	188	126	24	260	25.1	152	2	7	5	19	1.7	147	2.5	1.3	99	36	....	...	33	74.6
V	9,950	4,400	299 (325)	168 (100)	42	223 (425)	25.4	12,998	491	206	22	462	22.6	934	15	69	44	30	7.5	147	2.4	1.5	98	35	10.7	3.7	32	74.8
VI	9,283	4,160	300	156 (468)	39	162 (468)	27.5	10,000	467	230	22	634	22.6	382	6	66	23	36	3.6	153	1.9	1.2	102	35	9.8	3.1	38	73.4
VII	7,641	4,140	167	147 (750)	39	191 (750)	26.9	9,160	299	310	21	376	20.7	1105	13	100	37	133	6.4	151	4.0	1.4	104	31	....	3.1	33	74.6
VIII	10,200	4,160	193	149 (750)	40	198 (750)	28.3	10,670	321	479	15	791	20.8	581	3	93	20	51	4.2	151	4.1	1.4	112	25	9.5	3.2	40	74.8
IX	5,347	3,810	210	125	34	203	25.4	7,620	117	278	13	351	19.2	336	2	33	17	5	3.9	151	5.3	1.3	115	28	10.3	3.3	35	74.7
X	8,143	5,784	82	224	47	94	37.1	8,230	57	147	19	148	22.3	306	2	23	27	4	4.5	149	3.0	1.3	111	26	9.7	3.0	37	73.5
End of x	.....	.....	.....	.....	..	.....	....	.....	...	...	..	...	....	....	..	...	...	...	...	148	3.5	1.3	103	30	10.0	3.1	39	74.1

* Numbers in parentheses indicate supplements.

day with oral supplements. As was to be expected, there was a sharp rise in plasma potassium concentration and a marked uptake by the cells, amounting to a total of 1,020 mEq. in nine days. Urine K output increased in step-like fashion but was not sufficient to prevent the period IX, a markedly negative K balance and a fall in plasma potassium level to subnormal levels occurred, due to the continued high urinary excretion of potassium. Intracellular sodium increased and extracellular volume decreased. During period X, with potassium

TABLE IV
DERIVED BALANCE DATA

Period (3 days)	Total						Extracellular				Intracellular			
	Wt.	Na	K	Mg	Cl	N	H_2O	Na	K	Mg	H_2O	Na	K	Mg
	(kg.)	(mEq.)				(gm.)	(ml.)	(mEq.)			(ml.)	(mEq.)		
I	−0.7	− 17	− 82	−46	− 98	− 7.2	−0.55	− 83	+ 2	+2	−0.15	+ 66	− 83	−48
II	+0.3	+ 90	− 18	−11	+ 13	+ 1.0	+0.06	− 14	+ 0	−3	+0.24	+104	− 18	− 8
III	−0.1	+ 90	+ 6	− 6	+ 6	+ 4.8	+0.18	− 43	+ 5	+1	−0.28	+133	+ 1	− 7
IV	+0.2	+ 50	+ 15	+12	− 62	+ 0.9	−0.45	− 58	− 3	+2	+0.65	+109	+ 18	+10
V	−1.4	+118	− 7	−24	+ 8	− 4.7	−0.29	+ 10	− 5	−3	−1.11	+110	− 2	−21
VI	+1.2	−172	+328	− 6	− 40	+ 1.3	−0.65	−120	+10	+2	+1.85	− 52	+318	− 8
VII	+0.2	−145	+407	−19	+238	− 0.2	+1.43	+220	+16	+1	−1.23	−365	+391	−20
VIII	−0.1	−131	+327	+ 5	+105	+ 3.3	+0.57	+ 90	+16	0	−0.67	−221	+311	+ 5
IX	−1.2	+ 91	−186	+ 4	−153	+ 2.3	−0.86	−157	−29	−1	−0.34	+248	−157	+ 5
X	+0.6	+ 23	+ 54	+ 1	− 58	+10.3	+0.39	+ 30	+ 6	0	+0.21	− 7	+ 48	+ 1

continued rise of plasma K to supernormal levels.

Concomitant with potassium uptake, there was a markedly negative balance for sodium (−430 mEq.). During period VI both the extracellular and intracellular compartment contributed to this negative balance; however, during periods VII and VIII markedly negative intracellular sodium balance was accompanied by a positive extracellular balance. The extracellular volume decreased initially and then increased, whereas intracellular volume increased initially and then decreased later. Extracellular chloride gain was proportionately greater than extracellular sodium or water gain during periods VII and VIII, reflecting the correction of alkalosis and a rise in plasma chloride concentration.

With cessation of KCl therapy during intake of 72 mEq. per day, complete adjustment and equilibrium were noted.

Acute studies on urinary potassium excretion were made serially during a prolonged period of observation. (Fig. 6.) During the initial study (February 9, 1954), shortly after the first admission, filtration of potassium was markedly diminished due to the low glomerular filtration rate. Seventy-five per cent of filtered potassium was excreted and only 25 per cent reabsorbed by the tubules. With improvement in glomerular filtration to normal (March 30, 1954), the amount excreted into the urine decreased to 59 per cent of that filtered, 41 per cent being reabsorbed. Even after six months of normal GFR and Tm_{PAH} the amount of K reabsorbed was still only 68 per cent of the amount filtered.

During a sodium para-aminohippurate load (February 9, 1954), secretion of potassium by

the tubules was demonstrated, the amount excreted exceeding the amount filtered by 42 per cent. With improvement in glomerular filtration rate, sodium PAH load still produced an increase in potassium excretion, although this effect was less marked.

Studies on Alkalosis. A plasma carbon dioxide content of 42 mEq./L., initially found together with an arterial blood pH of 7.5, indicated a plasma CO_2 tension of 56 mm. of Hg, substantiating the presence of metabolic alkalosis. During the various periods of observation plasma CO_2 content was elevated when KCl supplements were not given and was usually, but not always, within the normal range when adequate supplements were administered. (Fig. 3.)

Development of alkalosis during potassium depletion was studied by analysis of the pH and titratable acidity of the urine. (Table v.) During the nine-day period of study on a 60 mEq. K/day diet, the plasma K fell from 3.5 to 2.5 mEq./L. and the plasma CO_2 content rose from 32 to 37 mEq./L. Urinary pH varied between the narrow range of 6.4 and 6.7 and the titratable acid varied in the subnormal range from 11.4 to 17.0 mEq. per day. Alkalosis thus appeared to develop in the presence of slightly acid urine, but with subnormal excretion of titratable acids.

Metabolic balance studies showed that during potassium therapy (VI, VII, VIII) potassium entered the cells (+1020 mEq.) and sodium left the cells (−637 mEq.) at a ratio of approximately 3:2. (Fig. 5.) Since the magnesium balance during this interval was only slightly negative (−23 mEq.), it might be postulated that approximately 360 mEq. of hydrogen ion emerged from the cells. Total extracellular bicarbonate content decreased by approximately only 40 mEq. during the period of high KCl therapy.

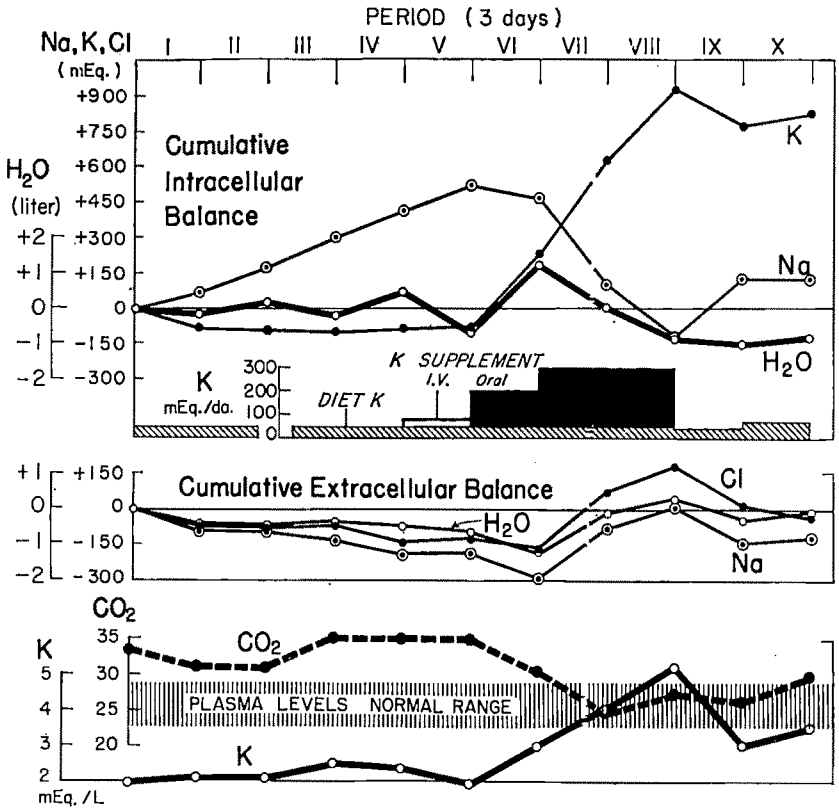


FIG. 5. Cumulative intracellular and extracellular balances of K, Na, Cl and H_2O, and plasma CO_2 and K concentrations during low and high intake of potassium.

Studies in Magnesium Metabolism. Serum magnesium levels, obtained periodically, were either low normal or, more frequently, subnormal. Magnesium sulfate supplements resulted in normal levels. (Fig. 3.)

Balance studies with magnesium intake of 12 to 14 mEq. per day showed a persistently

negative balance for twenty-one days, due mainly to large quantities of magnesium appearing in the stool. (Table III, Fig. 4.) Fecal loss was especially noticeable at the onset of the study and gradually decreased. The small amount of fecal magnesium and the apparent positive balance during period IV were due to temporary constipation, as evidenced by sudden release of magnesium in the stool during the following period. Positive balance was attained only after period VII, concomitant with replenishment of cellular potassium.

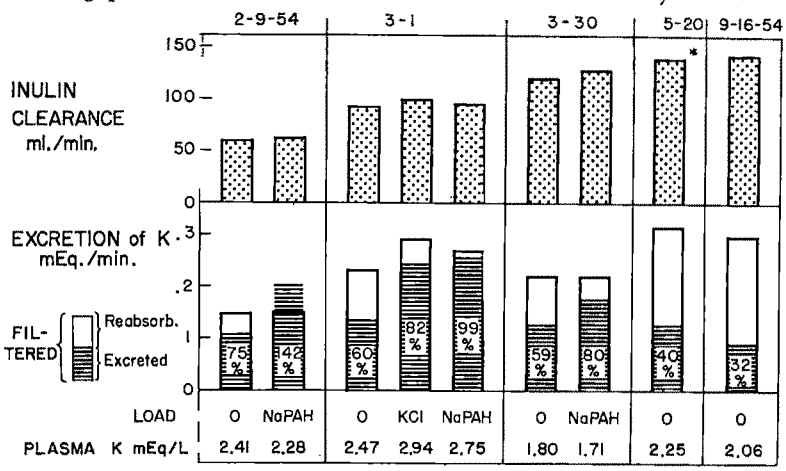


FIG. 6. Serial studies on urinary excretion of potassium. Asterisk indicates creatinine clearance × 0.9.

Acute urinary excretion of magnesium was studied after restoration of normal GFR and Tm_{PAH}, using clearance methods. (Fig. 7.) During the control period as much as 16 per cent of the amount filtered was excreted into the urine, 84 per cent being reabsorbed. Infusion of magnesium sulfate at the rate of 0.9 mEq. per minute resulted in rapid rise of serum magnesium levels and increase in the rate of excretion, due to increased filtration. The absolute quantity reabsorbed by the tubules remained relatively constant.

Studies Pertaining to Adrenal Cortical Activity. The tendency to retain sodium and to lose potassium suggested the possibility of some abnormality in adrenal cortical activity, even though clinical evidence of typical Cushing's syndrome or adrenogenital syndrome was lacking.

A glucose tolerance test after adequate carbohydrate intake for at least three days revealed a slight delay in return of the blood sugar to the control level. True fasting blood sugar was 67, one-half hour after ingestion of glucose—123, one hour—130, two hours—142, three hours—50, four hours—53 mg. per cent.

Analysis of thermal sweat showed the following values: Na 6.6, K 6.3, Cl 1.9, and Mg 0.5 mEq./L., indicating increased adrenal cortical activity.[17] Injection of desoxycorticosterone, however, failed to demonstrate a paradoxical increase in sodium excretion.

Results of the analysis of urinary steroids are given in Table VI.* Blue tetrazolium-reacting corticoids were increased significantly and the Porter-Silber (phenylhydrazine-reacting) corticoids were only slightly elevated above normal. The difference of these two fractions, representing the corticosterone-group of steroids was, therefore, significantly elevated. Likewise, the ratio of phenylhydrazine-reacting corticoids to

TABLE V

URINARY TITRATABLE ACID AND PH DURING DEVELOPMENT OF HYPOPOTASSEMIC ALKALOSIS

Date	Plasma (mEq./L.)		Urinary Titratable Acid (mEq./d.)	Urine (pH)
	K	CO_2		
Feb. 17	3.5	32	....	...
19	...	..	11.4	6.5
20	...	..	15.4	6.5
21	...	..	12.5	6.4
22	3.1	32	....	...
23	...	..	16.8	6.7
24	...	..	17.0	6.7
25	2.5	37	....	...
26	...	..	12.5	6.5

* The statement that derivatives of 17 hydroxycorticoids were increased, originally made in the abstract (*J. Lab. & Clin. Med.*, 44: 895, 1954), is an error, due to miscalculation of the data.

this difference was 0.56 or less, indicating a preponderance of corticosterone group of steroids in excess of the cortisone group of steroids. Excretion of 17-ketosteroid was normal, being fairly constant on three occasions, 6.6, 8.8 and 8.8 mg. per gm. creatinine. The Pettenkofer steroids were within normal limits.

above the normal and indicated excessive excretion of salt-retaining steroids.

COMMENTS

The fact that hypopotassemia could be demonstrated even after return of both glomerular filtration rate and capacity of the tubules to

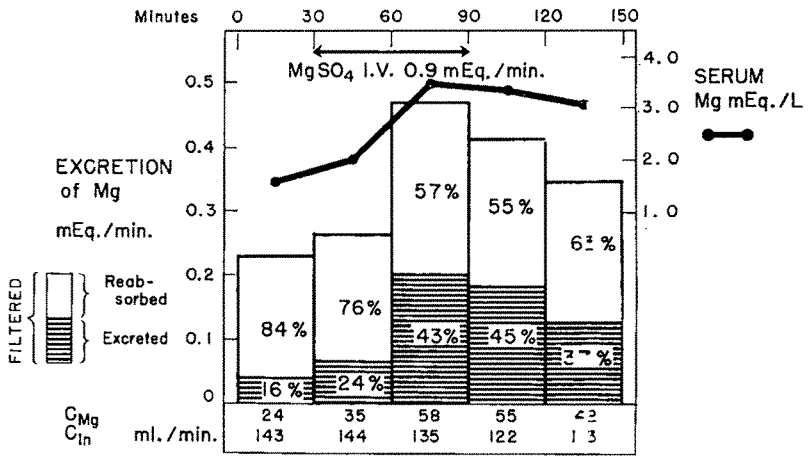


FIG. 7. Urinary excretion of magnesium before, during and after intravenous infusion of $MgSO_4$ at 0.9 mEq. per minute for sixty minutes.

Injection of 25 mg. of cortisone intravenously, complicated by a pyrogenic reaction, resulted in a marked eosinopenic response, from 280 to 40 per cu. mm.; a rise in urine volume, from 16 to 220 ml. per hour; a rise in urine Na concentration, from 13 to 78 mEq./L.; a rise in chloride, from 25 to 101 mEq./L.; and a slight rise in potassium, from 21 to 26 mEq./L. These changes suggested a paradoxic effect of exogenous cortisone given intravenously.

TABLE VI
URINARY CORTICOIDS*

	1–29	5–12	6–8	8–5
BTZ†	...	41.1	35.0	40.3
P-S‡	...	12.6	10.8	14.4
17 K-S§	6.6	....	8.8	8.8
BTZ minus P-S	...	28.5	24.2	25.9

* Expressed as mg. per gm. creatinine.
† Blue-tetrazolium reducing corticoids.
‡ Corticoids measured by Porter-Silber phenylhydrazine method.
§ Total 17-ketosteroids.

Bioassay of the urine obtained on two separate days, when KCl supplements had been discontinued, showed excretion of salt-retaining corticoids equivalent to 270 and 450 μg. DOCA per day. These values were definitely increased above the normal and indicated excessive excretion of salt-retaining steroids.

COMMENTS

The fact that hypopotassemia could be demonstrated even after return of both glomerular filtration rate and capacity of the tubules to excrete para-aminohippurate to the normal range indicated that intrinsic renal disease probably was not responsible for continued potassium loss. Low GFR and Tm_{PAH}, originally present, were perhaps due to severe depletion of tissue potassium.[18] Hypopotassemia *per se* had very little effect on GFR or Tm_{PAH}, as indicated by insignificant changes induced by KCl loading and continued improvement, in spite of acute lowering of plasma potassium levels, just prior to each testing. Although possible, it seems unlikely that the mild toxemia of pregnancy, which occurred eleven weeks prior to the initial study, was responsible for the decreased GFR and Tm_{PAH}. The renal plasma flow, as measured by para-aminohippurate clearance, remained constantly subnormal and undoubtedly reflected the underlying vascular hypertension which the patient had had for at least five years.

The polyuria manifested by this patient was ruled out as the cause of potassium loss by control studies on urine volume. The patient became extremely restless during restriction of water intake, although no significant changes in the plasma electrolyte concentrations could be detected.

It was apparent from metabolic studies that there was a tendency toward loss of potassium in the stool and urine. The gradual decrease

in this tendency during the first four periods on minimal intake of potassium suggested either (1) a gradual decrease in the stimuli for elimination of potassium, possibly because of hospital confinement, or (2) a metabolic lag period from higher intake prior to the onset of the study. The latter possibility was unlikely in view of the marked delay of potassium balance in reaching an equilibrium and the estimated K intake of only 15 to 20 mEq. per day more in the home than in the metabolic diet.

An intake of 50 mEq. of K per day should have been adequate for positive balance and correction of hypopotassemia, in view of the rapid uptake of K observed in two cases of catharsis-induced hypopotassemia studied by Schwartz et al.[18] The fact that this patient failed to retain potassium and correct her hyperpotassemia on a 50 mEq. K diet implies a definite abnormality in potassium metabolism.

Renal loss of potassium may be attributed either to excessive secretion or decreased reabsorption by the tubules. The net quantity "reabsorbed" by the tubules was only 68 per cent of the amount filtered, even after GFR and Tm_{PAH} had become normal This was distinctly below the normal of 80 per cent reported by Earle et al.[19]

Failure of the tubules to reabsorb K at a normal rate was apparently temporary, since immediately after the initial admission, when K depletion was undoubtedly the most severe, the urine-plasma potassium concentration ratio was as low as 1.8, a value usually not attained even in severe potassium depletion.[20]

Thus it appeared that some common factor was causing potassium loss from the bowels and the kidneys and that this factor had a self-limiting influence on tubular rejection (or excretion) of potassium.

Concomitant with the tendency toward potassium loss and hypopotassemia, there was a distinct tendency toward sodium retention. Random plasma sodium concentrations were usually but not always elevated above normal and metabolic studies showed retention of sodium during low K intake and loss of sodium during high K intake.

Calculations showed that these changes in sodium balance occurred primarily within the cells, affording an explanation for the absence of edema in the face of sodium retention. During K depletion and sodium retention, the extracellular fluid actually decreased; while during K repletion and sodium loss, the extracellular fluid increased, after a temporary unexplainable decrease for two to three days.

Development of alkalosis was undoubtedly related to potassium depletion[21,22] and since total titratable acids in the urine during potassium depletion were subnormal, extrarenal factors were probably at least partly responsible. Maintenance of an alkalotic state by persistent hypokaliemia must have been due to renal secretion of hydrogen ions.[23]

Metabolic studies revealed a large increment of fecal magnesium at the onset of hospitalization and a gradual decrease with confinement. Since the intake of magnesium was critically low (14 mEq./d.), the negative balance initially observed in this patient may have simply reflected an insufficient intake; however, the equilibrium attained during the last nine days of study (after increase of body potassium) suggested at least a temporary defect in magnesium metabolism.

Reabsorption of magnesium by renal tubules appeared to be somewhat impaired, as judged by the subnormal net reabsorption of filtered magnesium by the tubules. It would appear from data given by Heller et al.[24] that normally at least 95 per cent of the filtered magnesium is reabsorbed by the tubules. In the present case only 84 per cent was reabsorbed.

No symptoms could be ascribed to the hypomagnesemia *per se*, although the patient herself volunteered the information that headaches were alleviated by supplementary intake of magnesium. Muscular irritability, as judged by a positive Trousseau test, was apparently not caused by hypomagnesemia, since the test remained positive despite correction of the low serum magnesium level.

The entire picture of hypopotassemic alkalosis with retention of sodium, found in this patient, could be explained adequately by excessive secretion of adrenal corticoids; however, the absence of clinical features of Cushing's or adrenogenital syndromes suggested the possibility of oversecretion limited to the mineral corticoids. Chemical analysis of the urine indicated a preponderance of corticosterone or corticosterone-like hormones with unaltered or desoxy-17-carbon. Since corticosterone itself, when injected into rats, does not produce sodium retention,[12] it was most probable that the fraction found in the urine by chemical analysis and the salt-retaining component demonstrated by bioassay technic was not corticosterone but a corticosterone-like steroid

with unaltered 17-carbon. It is probable that this salt-retaining steroid was aldosterone.[25,26]

A case of hypopotassemia presumably due to excessive loss of potassium in chronic pyelonephritis has been reported by Cope and Garcia-Llaurado,[27] and Evans and Milne.[28] A significant amount of electrocortin (aldosterone), with salt-retaining activity, was isolated from the urine of this patient by paper chromatography.[27] It is possible that hypopotassemia developed in this patient and also perhaps in the patient reported by Earle et al.[19] as a result, in part, of oversecretion of salt-retaining, potassium-losing steroid. The renal dysfunction in these cases may have been secondary to severe chronic potassium depletion.[29]

A case presented by Conn* at the 1954 annual meeting of the Central Society for Clinical Research had clinical features identical with those of the case reported here and presented at the same meeting.[30] The increased urinary salt-retaining activity found in the present case corresponds with that found by Conn in his patient, and the term "primary aldosteronism" appears to be quite appropriate, especially in view of the studies made by Cope[27] in a case probably essentially identical with that of Conn and our case.

Paradoxic sodium diuresis was found by Conn two to three days after administration of either hydrocortisone or adrenocorticotrophic hormone, coincident with increase in excretion of 17-hydroxycorticoids. In the present case intravenous injection of cortisone resulted in a fall in the blood eosinophil count, and an immediate outpouring of urine with a marked rise in urinary concentrations of sodium and chloride and a slight rise of potassium. Such responses suggested opposing effects of the 17-hydroxy corticoids as against the action of the corticosterone-like salt-retaining steroid directly within the renal tubular cells.[31]

Whether or not aberration of magnesium metabolism, demonstrated by this patient, was due to oversecretion of corticosterone-like salt-retaining steroid (presumably aldosterone) cannot be determined; however, the tendency toward fecal as well as renal loss of magnesium suggests the presence of some humoral or hormonal factor operating at both loci. The studies on magnesium metabolism after injection of adrenocorticotrophic hormone have been inconclusive.[32] This may be due to the fact that ACTH appears to have very little effect on the secretion of the specific mineralocorticoid.[33]

SUMMARY

A case of spontaneous hypopotassemia, hypomagnesemia, alkalosis and tetany is presented. Metabolic studies indicated excessive excretion of potassium and magnesium in the urine and stool, and concomitant retention of sodium. Potassium supplements resulted in uptake of potassium and loss of sodium from the cells. Alkalosis was attributed to potassium depletion. Evidence is presented that organic renal disease and polyuria *per se* were probably not the primary causes of potassium loss.

Studies of the urine revealed increased excretion of corticosterone-like steroid by chemical tests and increased salt-retaining corticoid by the bioassay method. The entire picture could be explained by oversecretion of corticosterone-like mineralocorticoid, presumably aldosterone.

Acknowledgment: The authors are indebted to Drs. William Q. Wolfson, Harry W. Hays, Vito De Fillipis, William B. Maddock and David Young for their valuable assistance, and to Mrs. Maxine Adams, Mrs. Nancy Davenport and Mrs. Juanita Maxwell for their technical aid.

ADDENDUM

On February 28, 1954, the patient was operated upon and the left adrenal gland, containing a cortical tumor, 2.5 cm. in diameter and 6.5 gm. in weight, was removed. The normal portions of the gland appeared atrophic. Microscopic examinations showed an active adrenal cortical tumor tissue, resembling the cells of both zona glomerulosa and zona fasciculata. Bioassay of the tumor extract revealed a salt-retaining factor equivalent to 27 μg. of DOCA per gm. of tissue. Dr. Albert Wettstein, of Basle, Switzerland, graciously analyzed the extract chemically and found 1.4 μg. of aldosterone and 33 μg. of corticosterone per gm. of tumor tissue. Preoperative twenty-four-hour urine contained 16.8 μg. of aldosterone. These values agreed well with the bioassay results.

Subsequent to surgery, the blood pressure and the biochemical abnormalities have become normal, implicating the tumor etiologically.

A tumor was also found in Conn's case.[34]

* See footnote on page 976.

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